"EVALUATION OF EXPRESSION OF HER2/NEU IN GASTRIC CANCER AND ITS SIGNIFICANCE AS A PROGNOSTIC FACTOR IN GASTRIC ADENOCARCINOMA"

DISSERTATION SUBMITTED TO

THE TAMILNADU DR.M.G.R. MEDICAL UNIVERSITY

CHENNAI

in partial fulfilment of

the requirements for the degree of

M.D. (PATHOLOGY)

BRANCH - III

TIRUNELVELI MEDICAL COLLEGE HOSPITAL

TIRUNELVELI

APRIL-2015

CERTIFICATE

This is to certify that this Dissertation entitled " **EVALUATION OF EXPRESSION OF HER2/NEU IN GASTRIC CANCER AND ITS SIGNIFICANCE AS A PROGNOSTIC FACTOR IN GASTRIC ADENOCARCINOMA"** is the bonafide original work of **Dr. M.KAVITHA** during the period of her Post graduate study from 2012 – 2015, under my guidance and supervision, in the Department of Pathology Tirunelveli Medical College& Hospital, Tirunelveli, in partial fulfillment of the requirement for M.D., (Branch III)in Pathology examination of the Tamilnadu Dr.M.G.R Medical University will be held in April 2015.

The DEAN

Tirunelveli Medical College,

Tirunelveli - 627011.

CERTIFICATE

I hereby certify that this dissertation entitled **"EVALUATION OF EXPRESSION OF HER2/NEU IN GASTRIC CANCER AND ITS SIGNIFICANCE AS A PROGNOSTIC FACTOR IN GASTRIC ADENOCARCINOMA"** is a record of work done by **Dr. M.KAVITHA ,** in the Department of Pathology, Tirunelveli Medical College, Tirunelveli, during her postgraduate degree course period from 2012- 2015.This work has not formed the basis for previous award of any degree.

Dr.K.Shantaraman, M.D.,
Professor and HOD of Pathology,
Department of Pathology,
Tirunelveli Medical College,
Tirunelveli -11

TIRUNELVELI MEDICAL COLLEGE
TIRUNELVELI,
STATE OF TAMILNADU, INDIA
PIN CODE:627011
Tel: 91-462-2572733, 2572734 Fax: 91-462-2572944
Under the Directorate of Medical Education, Government of Tamilnadu.
Estd:1965

Institutional Ethical Committee

Certificate of Approval

This is to certify that the Institutional Ethical Committee of this College unanimously approves the Thesis /Dissertation/ Research Proposal submitted before this committee by Dr.M.Kavitha, Post Graduate in Pathology, Department of Pathology, Tirunelveli Medical College /Hospital, Tirunelveli titled **"EVALUATION OF EXPRESSION OF HER2/NEU IN GASTRIC CANCER AND ITS SIGNIFICANCE AS A PROGNOSTIC FACTOR IN GASTRIC ADENOCARCINOMA"** registered by the IEC as 299/PATHO/IEC/2012 dated. 14.12.2012. The Investigator is hereby advised to adhere to all the stipulated norms and conditions of this ethical committee.

Issued on this Date

14.12.2012

Under Seal

TIRUNELVELI MEDICAL COLL. TAMILNADU INDIA TIRUNEL

Secretary

Secretary,
Ethical Committee,
Tirunelveli Medical Coll
Tirunelveli-11.


Turnitin Document Viewer - Google Chrome
EVALUATION
turnitin
14%
Match Overview
1 1%
2 1%
3 1%
4 1%
5 1%
6 1%
7 <1%
8 <1%
9 <1%

DECLARATION

I solemnly declare that this dissertation titled**" EVALUATION OF EXPRESSION OF HER2/NEU IN GASTRIC CANCER AND ITS SIGNIFICANCE AS A PROGNOSTIC FACTOR IN GASTRIC ADENOCARCINOMA"** submitted by me for the degree of M.D, is the record work carried out by me during the period of 2012-2015 under the guidance of **Prof. Dr.K.Shantaraman M.D.,** Professor and HOD of Pathology, Department of Pathology, Tirunelveli Medical College, Tirunelveli. The dissertation is submitted to The Tamilnadu Dr. M.G.R. Medical University, Chennai, towards the partial fulfilment of requirements for the award of M.D. Degree (Branch III) Pathology examination to be held in April 2015.

Place: Tirunelveli
Date:

Dr.M.KAVITHA,
Postgraduate Student,
M.D Pathology,
Department of Pathology,
Tirunelveli Medical College,
Tirunelveli.

ACKNOWLEDGEMENT

I take immense pleasure at this opportunity to acknowledge all those who have helped me to make this dissertation possible.

I express my heartfelt thanks to the **Dean, Tirunelveli Medical College,** for permitting me to undertake this study. I express my profound sense of gratitude to **Dr.K.Shantaraman MD.,** Head of Department of Pathology and my respected guide, Tirunelveli Medical College, Tirunelveli ., for his unstinted guidance and motivation.

I thank **Dr.S.Vallimanalan, M.D., Dr.K.Swaminathan, M.D., Dr. J. Suresh Durai, M.D., Dr. Arasi Rajesh, M.D.,** Professors of Pathology for their constant support and encouragement.

I profusely thank all the other faculties and my postgraduate colleagues for their valuable support.

I also sincerely thank the Technicians and other members of the Department of pathology and the Central Diagnostic Lab for their kind cooperation.

I thank all my family members for their encouragement and support during this study.

ABBREVIATIONS

GEJ	-	GASTRO ESOPHAGEAL JUNCTION
CAG –A	-	CYTOTOXIN-ASSOCIATED GENE A
UICC	-	INTERNATIONAL UNION AGAINST CANCER
H.PYLORI	-	HELICOBACTER PYLORI
TP53	-	TUMOUR PROTEIN
CDH1	-	CADHERIN-1GENE
HER2	-	HUMAN EPIDERMAL GROWTH FACTOR RECEPTOR 2
MLH1	-	MUTL HOMOLOG 1
MSH2	-	MUTS HOMOLOG 2
MSH6	-	MUTS HOMOLOG 6
PMS2	-	POSTMEIOTIC SEGREGATION INCREASED 2
MSH3	-	MUTS HOMOLOG 3
SMAD	-	MOTHERS AGAINST DPP HOMOLOG
BMPRIA	-	BONE MORPHOGENETIC PROTEIN RECEPTOR, TYPE IA
PTEN	-	PHOSPHATASE AND TENSIN HOMOLOG
STK11	-	SERINE/THREONINE KINASE 11
LKB1	-	LIVER KINASE B1
BRCA1	-	BREAST CANCER 1
BRCA2	-	BREAST CANCER 2
HNPCC	-	HEREDITARY NONPOLYPOSIS COLORECTAL CANCER

FAP	-	FAMILIAL ADENOMATOUS POLYPOSIS
PJS	-	PEUTZ-JEGHERS-SYNDROME
GKS3	-	GLYCOGEN SYNTHASE KINASE 3
TCF/LEF	-	TRANSCRIPTION FACTOR/ LYMPHOID ENHANCER BINDING FACTOR
CIN	-	CHROMOSOMAL INSTABILITY
RB	-	RETINOBLASTOMA GENE
nm23	-	nucleoside diphosphate kinase 1
CpG	-	—C—phosphate—G—
CA72-4	-	CARBOHYDRATE ANTIGEN 724
DAB	-	3,3'-DIAMINOBENZIDINE
H&E	-	HEMATOXYLIN AND EOSIN
MUC1	-	MUCIN 1
PAS	-	PERIODIC ACID–SCHIFF
MPC	-	MICRO PAPILLARY CARCINOMA
EBV	-	EPSTEIN–BARR VIRUS
GCLS	-	GASTRIC CARCINOMA WITH LYMPHOID STROMA
AFB	-	ACID- FAST BACILLI
CEA	-	CARCINOEMBRYONIC ANTIGEN
DNA	-	DEOXYRIBONUCLEIC ACID
LOH	-	LOSS OF HETEROZYGOSITY

MDM2	-	MOUSE DOUBLE MINUTE 1 PROTEIN
miRNAs	-	MicroRNAs
hMLH1	-	human mutL homolog 1
hMH2	-	human mutS homolog 2
TGF	-	TRANSFORMING GROWTH FACTOR

CONTENTS

ABSTRACT

Gastric carcinoma is the second common cause of cancer related worldwide deaths. The HER-2/neu is the Human epidermal growth factor receptor-2 and it is a gene localized on chromosome 17q21 that encodes a growth factor receptor like molecule with tyrosine kinase activity and has a structure similar to that of epidermal growth factor receptor. Its expression has been detected in several human cancers and is believed to be associated with poor prognosis, aggressive biological behavior and metastatic potential. The present study is to evaluate the presence of HER-2/neu in gastric cancers of all histological types. IHC is an excellent technique to detect HER-2/neu expression in Gastric carcinoma. Their prevalence in gastric cancer ranges from 9-38% in most studies. This study included 50 cases of gastric carcinoma and these were subjected to immunohistochemical staining for HER-2/neu oncoprotein. Out of 50 cases , only 8 cases of Gastric adenocarcinoma (16%) were found to be positive for HER-2/oncoprotein. In this study Her2 neu expression is more in Oesophageal gastric junctional tumours & corpus location of stomach tumours (35.3%) compared to Pylorus-Antral tumours. And also we observed Her2 neu expression in patients with signet ring cell carcinoma (40%) compared to Non signet ring cell carcinoma.

KEYWORDS: Gastric Carcinoma, IHC, HER-2/neu

INTRODUCTION

Every year 3 million new cases of gastrointestinal tract cancers are reported world wide[1]. Gastric carcinoma is the second common cause of cancer related deaths and it was responsible for more than 738,000 cancer deaths in 2008[2] . Gastric cancers are the second most common cancers in women and fourth most common in men[3,4]. Gastric cancer is associated with a higher number rate of cancer related deaths, more than other common malignancies like colon and breast[5].Predominant gastric malignancies (95%) are epithelial in origin[6]. The frequency of the gastric cancer varies according to the geographic locations, diet and lifestyle habits. Japan and South Korea and China have the highest incidence rates, while low incidence rates are reported in north America and Africa[3,7,8,9].

Gastric cancer is very rare below the age of 30 years. The age related incidence rises sharply and peaks in the age group of 60-80 years[10]. In India, gastric cancers are common in the age group of 45 to 55 years in North India and 35 to 55 years in South India. Males are more prone to gastric cancer than females[3]. In India, the frequency of gastric cancer is higher in the south and north eastern states.

The state Mizoram reported an age adjusted incidence rate of 50.6 and 23.3 for male and female respectively[11]. There is a constant

decline in incidence and mortality rates of gastric carcinoma in industrialised countries.

Surgical removal of the primary tumour is curative in stage I cancers, but for more advanced stage gastric cancers, surgery combined with postoperative chemotherapy and or radiation is used. But the survival rates are still low[12]. Advanced gastric cancers are strongly associated with poor outcome with a median survival of patients with metastasis at 7 to 10 months from initial diagnosis [13].

TNM stage is a dependable prognostic tool in gastric carcinoma patients, but patients with similar TNM-stages been observed to have different clinical outcomes. Even with aggressive chemotherapy, recurrences are common in these patients, even in early stage of disease and hence the average five year survival remains between 30 to 50%14 . Hence the need of new and non traditional methods of prognostication of gastric cancers. The present regimens of chemotherapy give unsatisfying results, hence a need for a therapy for a therapy regimen that is tailored to the individual patient based on the molecular pathways associated with tumour cell growth, proliferation, invasion, and metastasis and that which is specific to tumour-related molecular targets. One such example is the drug targeting the human epidermal growth factor receptor2 (HER2) in patients with breast cancer.

The prognostic significance and prevalence of HER2/neu in patients with gastric carcinoma is less established than in breast cancer. Their prevalence in gastric cancer ranges from 9-38% in most studies[15], although values as low as 2.5% to as high as 91% have been reported[16]. Some studies has identified HER2/neu as a poor prognostic factor for survival, some have reported the same as a positive prognostic factor and others have failed to establish the relationship[15-18]. Hence the reports of the correlation of HER2/neu and Gastric cancers is conflicting and hence needs more study.

The prognostic significance of HER2/neu in gastric carcinoma is still under study. Current best practice for assessing HER2 in gastric cancer involves immunohistochemistry (IHC) followed by molecular analysis of *HER2/neu* gene amplification, by fluorescent in situ hybridization (FISH)[19]. This methodology was not widely accepted until 2010.In this study, the expression of HER-2/neu in gastric cancer is studied by immunohistochemistry. The correlation between HER-2/neu expression and the clinicopathological parameters of the patients are analysed.

AIMS &OBJECTIVES

1. To Assess the expression of HER2/*neu* in gastric cancers of all histological types.
2. To describe the relationship between HER2/*neu* expression in gastric adenocarcinoma and the Clinicopathological data of these patients.

REVIEW OF LITERATURE

EMBRYOLOGY

The stomach develops as a fusiform dilation of the foregut at the 4mm stage in week[21]. The primordium soon enlarges and broadens ventro-dorsally. Its position and appearance changes as a result of the different rates in the growth, as well as changes in position of the surrounding organs. The positional changes are explained by the antero-posterior axial rotation of the stomach. The stomach carries out a 90 clockwise rotation around the longitudinal axis, causing its right side to face posteriorly and its left side anteriorly[20]. Antero-posterior axis rotation displaces the cephalic portion towards left and slight downward and the pyloric part of the stomach towards right and upward, resulting in the future duodenum coming to be retroperitoneal.(Figure1)

The dorsal part of the stomach grows faster than the ventral part which results in formation of the greater and lesser curvatures of the stomach. Stomach is attached to the posterior body wall by dorsal mesogastrium in this stage. Longitudinal axial rotation pulls the dorsal mesogastrium towards left and helps in the formation of lesser sac.

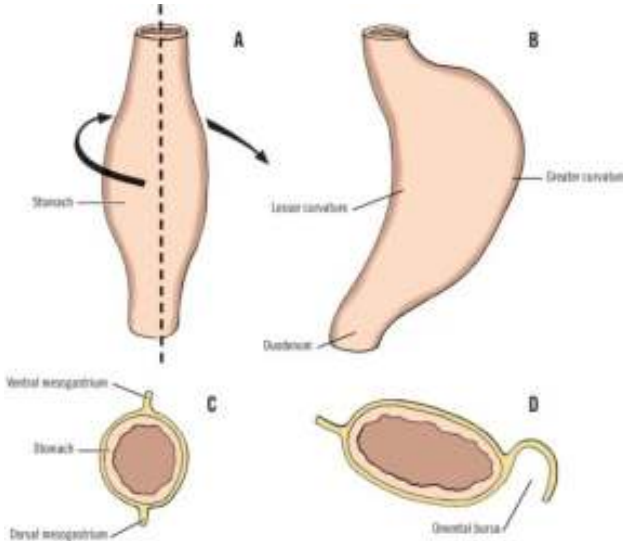


Figure 1. Embryological development of stomach

As the embryo lengthens, the distal part of the septum transversum becomes the mesogastrium of the stomach, which attaches the duodenum and stomach to the ventral wall of the abdominal cavity. Then stomach assumes its final position, in which greater curvature faces downward, and the lesser curvature faces to the right and upward .

ANATOMY

The stomach is divided grossly into four regions: cardia, fundus, body (corpus) and pylorus. It extends as a J shaped loop from the distal end of oesophagus at the level of 11^{th} thoracic vertebrae, to just right of the 1^{st} lumbar vertebra inferiorly, where it connects to the duodenum. The stomach begins from the GEJ, which is the proximal part of the gastric folds and ends at the pylorus, where the circular smooth muscle thickens to form the pyloric sphincter. The concavity towards right,

forms superomedial margin is termed as lesser curvature and the convexity of the left, outer curve forms inferomedial margin, which considered to be greater curvature. The angle at which the pylorus narrows before the gastroduodenal junction termed as incisuraangularis.

Topographically, the stomach is divided into following five regions (Figure 2).

1. The cardia is a narrowest (0.1 to 0.4 cm in length) and macroscopically indistinct portion of the stomach which is located immediately distal to the GEJ.
2. The fundus is known as dome of the stomach which is just beneath the diaphragm and extends superolateral to the oesophagogastric junction.
3. The body of the stomach represents the largest portion of the stomach and it comprises the proximal 2/3rdof the remainder.

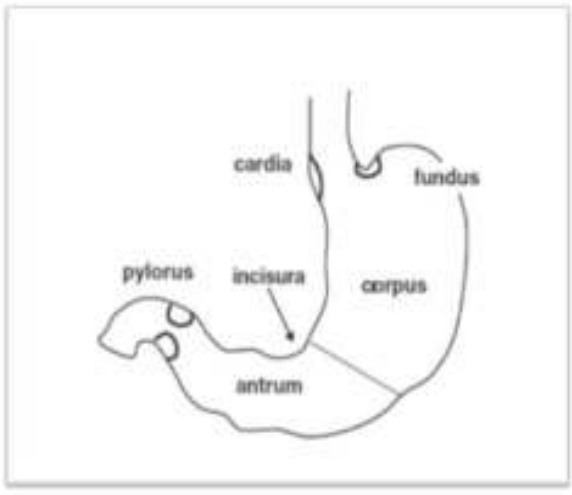


Figure 2.Topography of the stomach

4. The part of stomach which is distal to the incisura is termed as pylorus antrum. It is distinguished from the proximal duodenum by the pyloric sphincter.
5. The pylorus has no easily visualised landmarks, but is easily palpated as a ring of muscle which separates the stomach and duodenum.

The gastric wall consists of mucosa, submucosa, muscularispropria, and serosa. The interior surface of the gastric mucosa shows coarse rugae, which flattens when the stomach gets distended. Rugae or folds are more prominent in the proximal stomach.

Blood supply and lymphatic supply:

The blood supply of the stomach originates from the celiac axis, hepatic artery, and splenic artery. The stomach is richly vascularised organ, with contributions from five major sources (Figure 3).

1. The right gastric artery , a branch of proper hepatic or common hepatic artery, which anastomoses with left gastric artery and supplies the distal portion of lesser curvature.
2. The left gastric artery, a smallest branch of celiac axis, which supplies the cephalad portion of the lesser curvature.
3. Right gastroepiploic artery supplies antrum and lower part of body, which originates from gatroduodenal artery.

4. Left gastroepiploic artery supplies upper corpus which originates from splenic artery.
5. A series of short gastric arteries from splenic artery, which supplies proximal stomach.

The right and left gastric veins drains into the portal vein, left gastroepiploic vein into splenic vein and right gastro epiploic vein into superior mesenteric vein.

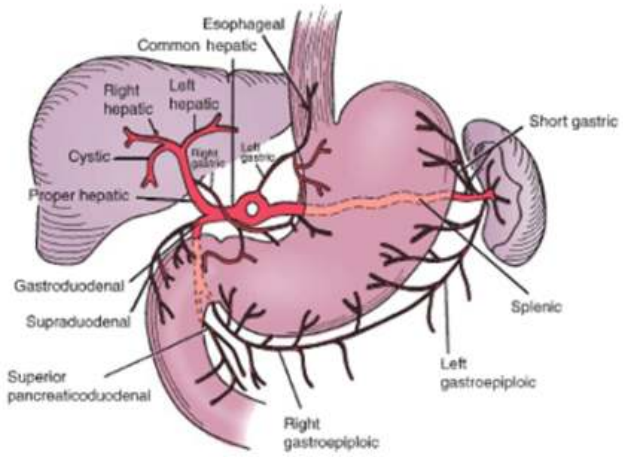


Figure 3. Branches of celiac trunk

Lymphatic drainage:

Lymphatic drainage pathways run in close proximity to the arterial supply (Figure 4)[22].The lymph vessels drain into four major areas, according to the following scheme:

1. Upper lesser curvature: left gastric nodes and para cardinal nodes via superior gastric group.

2. Pylorus and caudal portion of lesser curvature: right supra pancreatic nodes via suprapyloric group.
3. Cephalic portion of the greater curvature: splenic and left gastroepiploic nodes via pancreaticosplenic group.
4. Caudal portion of greater curvature: right gastroepiploic nodes via Subpyloric group.

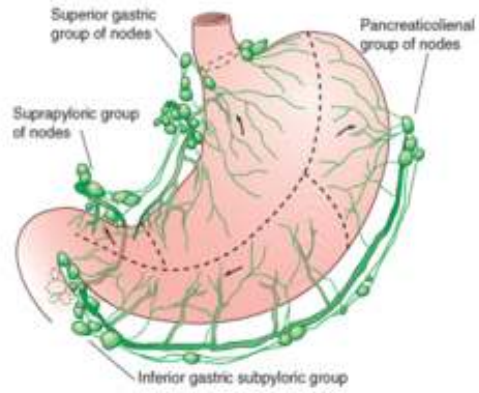


Figure 4 Lymphatic drainage of the stomach.

Eventhough these lymph nodes drains different region of the stomach, it remains widely accepted that gastric carcinoma may metastasize to any of the nodal group irrespective of their location.

Innervation:

The extreme innervation of the stomach is both sympathetic through celiac plexus and parasympathetic through the vagus. The anterior (left) vagus gives off the hepatic branch to liver and continues as the anterior nerve of latarjet along the lesser curvature (Figure 5)[23].

The right vagus gives a branch off to celiac plexus and tending to supply posterior surface of the stomach. In contrast, sympathetic nerve supplies arise from D6-D10 travelling through the splanchnic nerve to the celiac ganglion and spreading thence to stomach.[24]

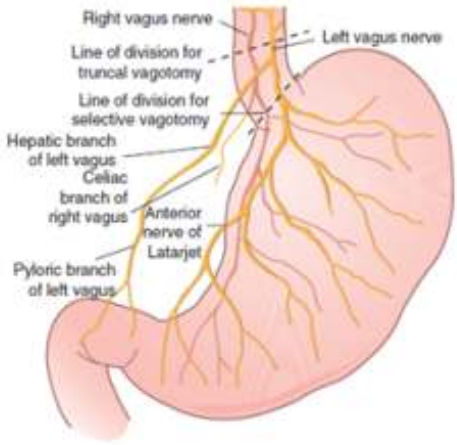


Figure 5.Vagal innervation of stomach

Histology:

The organization of the gastric mucosa is similar in all regions of the stomach[25]. Gastric mucosa consists of columnar epithelia, which invaginated from the surface form the gastric pits (foveolae). Gastric glands extends from the pits into the mucosa, which vary in the structure and function in the three regions.

The lengths of the glands and pits also exhibit variability in the three regions of the stomach. The cardiac portion of the stomach extends a short distance from the junction with the oesophagus to the fundus, it usually extends for 5–30mm distal to cardio-oesophageal junction.[25]It is

characterized by equal length of foveolae and glands and it has loosely packed mucus-secreting cells. Surface mucous cells line the foveolae, which project inwardly from the surface of the organ which secretes a protective coat of mucus that prevents the mucosa from being digested by the acidic environment of the lumen.

The fundus part of the stomach which lies above an imaginary, horizontal line through the oesophageal orifice and the body of the stomach, which is below that line. The fundus and main body of the stomach are similar in their histologic structure. Body and fundal mucosa consists of tightly packed straight tubular glands which arranged perpendicular to the surface (Figure 6), that synthesise and secrete gastric juice. The gastric pits form superficial zone which occupies 25% of the total thickness and each has between one and seven gastric glands opening into it.

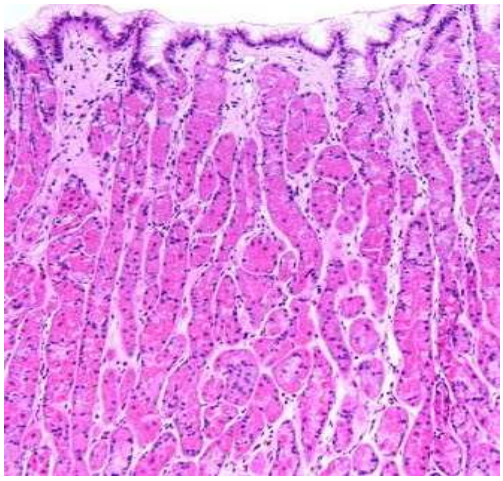


Figure 6. Normal body mucosa

The gastric glands of the body mucosa exhibit four types of cells. They are mucous neck cells, chief or peptic cells (zymogenic cells), acid secreting parietal or oxyntic cells and endocrine cells.[25,26]The rest of the wall is composed of the muscularis mucosae, submucosa, muscularis propria, and serosa, as are the other regions of the stomach. Below the muscularis mucosae is the connective tissue of the submucosa. Fibroblasts and other cellular components of connective tissue, as well as blood vessels and nerve fibers are found in the submucosa. Beneath the submucosa, the external smooth muscle layers are arranged in indistinct inner oblique, middle circular, and outer longitudinal patterns. Outer longitudinal, inner circular, and innermost oblique layers are the three layers of the muscularis externa. The serosal layer of the stomach is continuous with the serosal lining of the peritoneal cavity. Pyloric mucosal zone occupies distal 3-4cm of the stomach, in contrast to the simple straight glands of the fundus and body, the pyloric glands are coiled tubules, some of it branched which occupies 50% of the thickness of the pyloric mucosa, and separated by upgrowths of muscularis mucosae (Figure 7).

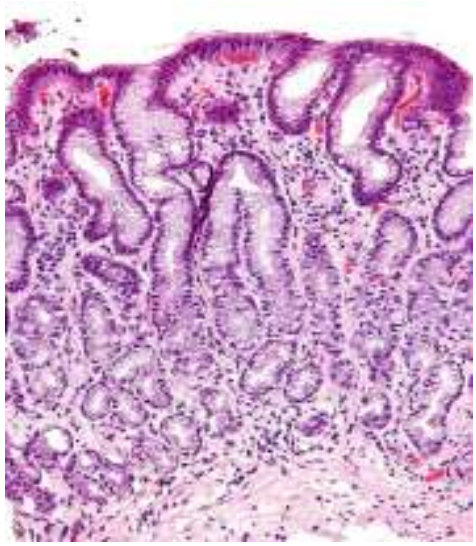


Figure 7 Normal Antral mucosa.

The pyloric glands are lined almost exclusively by mucus-secreting cells, which are similar to the neck mucous cells of the gastric body and fundus. In addition to mucus-secreting cells, pyloric glands also contain cells of the diffuse neuroendocrine system that produce the peptide hormone gastrin, which promotes acid secretion by the parietal cells.

PHYSIOLOGY

The principle function of the stomach is digestion of food and absorption as it is propulsed through small intestine. The initial period of digestion require several hours while they undergo physical breakdown of the bolus of food into a semisolid mass known as

chyme. The strong muscular action of the stomach mechanically reduces food, and the action of enzymes secreted by the cells of the stomach chemically digests proteins. In the stomach, the food suffers significant physicochemical changes which as a result of the secretor and motor activity of the stomach. The mechanical activity of the stomach consists of the presence of peristaltic waves, which helps in gastric evacuation, contractile activity of the stomach being continuous during both digestive and inter digestive periods. The gastric glands secretes gastric juice, which made up of 99% water and 1% inorganic substances - hydrochloric acid, mainly, and organic substances, mucin and gastric enzymes: pepsin, rennin, gelatinases, lipase. The endocrine secretion of the stomach is represented by the secretion of gastrin, somatostatin, histamine, or leptin. The functions and the cells lining the glands vary according to the region of the stomach (Table 1)

Table 1 Gastric cell types, location, and function

Cells	Location	Function
Parietal	Body	Acid secretion and intrinsic factor
Mucus	Body, Antrum	Mucus
Chief	Body	Pepsin
Surface epithelial	Diffuse	Mucus. Bicarbonate
Enterochromaffin- like	Body	Histamine
G	Antrum	Gastrin
D	Body, Antrum	Somatostatin
Gastric mucosal interneurons	Body, Antrum	Gastrin-releasing peptide
Enteric neurons	Diffuse	Calcitonin gene- related peptide
Endocrine	Body	Ghrelin

EPIDEMIOLOGY:

The incidence of the gastric carcinoma varies according to their geographical location. The incidence of gastric cancer in India is less compared to worldwide incidence. The age – adjusted rate (AAR) of gastric cancer among urban registries in India is 3.0 -13.2 compared to

worldwide AAR 4.1- 95.5.[27-30] The age – adjusted incidence rate of stomach cancer in males varies widely among registries, highest being 11.2/ 100,000 in Chennai compared to 1.6/ 100,000 in Bhopal[31]. In India highest incidence rates are found in south and northeast regions. In 2010, nationally representative survey found 556,400 number of deaths occur due to cancer, in which gastric cancer shows 12.6% mortality rate and considered to be the second most widely known cancer.[32] Gastric cancer is unusual below the age of forty years and climbs thereafter and attain peak in the seventh decade of life.[33]

In India male to female ratio of the incidence was 2.3:1. the median age for men was 58 years and for women 57 years.[34] Five-year survival for gastric cancer is nearly 20%. Survival rates are higher in countries which have effective screening programs that lead to early detection and where distal cancer predominates.[35]

PATHOBIOLOGY OF GASTRIC CARCINOMA:

The pathogenesis of gastric carcinoma involves multiple risk factors. More than 60% of gastric cancer cases have been contributed to H.pylori infection. In addition to infections, other conditions such as diet, cigarette smoking, male gender, genetic factors, nitrates, chemicals, socioeconomic status and other pathological conditions in stomach can also contribute to tumoriogenesis. Gastric carcinogenesis is a multi-step

process, with the following sequential stages: progression from the normal gastric mucosa through chronic gastritis which gradually results in atrophy, then intestinal metaplasia and dysplasia.[36]

RISK FACTORS ASSOCIATED WITH GASTRIC CARCINOMA:

A.Helicobacter pylori infection

Warren and marshall discovered the H.pylori organism 1982[37]. It is a Gram- negative bacteria found in the normal stomach mucosa that can be remain alive and proliferate in the stomach, and it has been acknowledged as an significant risk factor for gastric carcinoma[38,39]. Higher prevalence is much more common in developing countries when compared with developed countries . H.pylori is usually acquired during childhood and most frequently run in families with low socio economic status[40]. It has been classified as group I carcinogen by WHO. Approximately 50% of world population is infected by H.pylori[41] and it is associated with increased risk of developing gastric cancer[42].NotAll the infected individuals prone to develop pathological lesions. (Figure8). Thus, the factors determining gastric cancer development remain largely unknown.

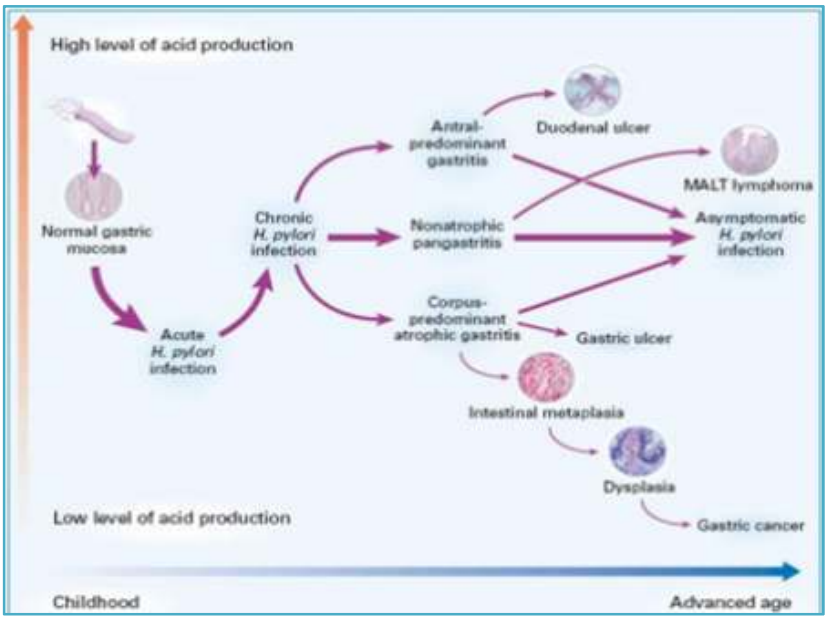


Figure 8. clinco pathological outcome associated with H.Pylori infection

H.pylori infection is associated with both diffuse and intestinal type gastric cancer. It shows a high degree of genetic heterogeneity, so several virulence factors of the bacterium also plays a role, which establish the outcome of the infection. CagA-positive strain produces higher level of IL 8, it often elicits intense inflammatory reaction which are associated with raised risk of gastric carcinoma. Compared with CagA-negative strain, CagA-positive bacteria induces severe inflammation and have been associated with greater risk for peptic ulcer disease, as well as preneoplastic and neoplastic lesions[43]. Smoking act as an additive factor which significantly raises the risk of gastric carcinoma whiich is associated with CagA positive H. pylori infection [44]. H. pylori infection is very unusual with adenocarcinoma of the gastric cardia[45].

Other important bacterial virulence factors are VacA, adhesins. The ultimate mechanism by which H. pylori induces stomach cancer remains unknown. Eradication of Helicobactor infection seems to be decrease the incidence of gastric carcinoma[46]

B .Dietary and lifestyle factors

Diets rich in salty foods such as salted meat, dried fish, smoked fish and pickled foods which favours intra luminal formation of genotoxic agents and it has been found to be related with development of gastric carcinoma. Food rich in citrus fruits and vitamin C, vegetables are inversely associated with risk of developing gastric adenocarcinoma[47,48]

A high salt intake act as an irritant to the stomach mucosa which elicit mucosal damage and increases epithelial cell proliferation. Numerous studies have found smoking has been associated with gastric carcinoma [49,50]. High alcohol consumption also can also act as a risk factor for gastric cancer[51], but this has not been the case in all studies [50].Increased risk was also observed among the tobacco chewers[52]. Hyperglycaemia add significant risk for gastric cancer, especially among H. pylori positive cases[53]. Capsaicin may also act as a risk factor for gastric carcinoma[54]. Adequate intake of vitamin C is associated with lower gastric cancer risk[55].

A study in Colombia showed that after 6 years of dietary supplementation with vitamin C and beta-carotene, partial regression of gastric precancerous lesions was observed[56] .

Low socioeconomic status and its correlates increased the risk of developing gastric cancer[49,50].

C. Family history and genetic conditions

Family history

First- degree relatives of the affected person are nearly three times higher risk of developing gastric carcinoma compared with general population[57]. CagA positive H.pylori infection associated with family history of gastric cancer and it has 16 – fold risk of developing non cardia gastric cancer[58].

Genetic conditions

Most gastric cancers are sporadic in nature. But 8-10% of gastric cancer cases are associated with inherited genetic components [59]. Table-2 summarises common gastric sporadic neoplasms and their genetic alterations.

Table 2 sporadic neoplasm and their genetic alterations

Tumour	Genes Involved	Common Genetic Alterations
Gastric Carcinoma	P53, CDH1,c-erbB-2, cmet, MLH1,MSH2,MSH6,PMS2, MSH3,Loss or gain of 3p,4,5q,6q,9p,17p	Pointmutation, duplication, deletions, Insertions or Gain

Hereditary diffuse gastric carcinoma

Familial diffuse gastric cancer usually common in younger age group[60]. It is an autosomal dominant inheritable condition.75% of gastric cancer tumours associated with germ line mutations of E-cadherin (CDH1) gene which forms truncated proteins[61].The age of onset and diagnosis is in-between 14 to69 years. These hereditary diffuse gastric carcinomas usually manifests as diffuse or poorly differentiated carcinomas with an extensive infiltrative growth pattern, often contains signet-ring cells[62]. In Addition, methylation of the CDH1 gene promoter isalso results in hereditary diffuse gastric carcinoma[63] . Patients with inherited genetic disorders, such as inherited BRCA1 and BRCA2 genes and HNPCC may also associate with increased risk of developing gastriccarcinoma[64]

Table-3 summarises common Inherited Genetic syndrome and their genetic alterations.

D Gastric surgery

The increased risk of developing gastric cancer in the gastric stump of patients who undergone previous gastric surgery[65],particularly bilrothII gastrectomy operation which increases bile reflux. The occurrence of bile reflux in turn promotes gastric carcinogenesis.[66]

Table 3 Inherited Genetic syndrome &their genetic alterations

Syndrome	Gene Involved	Genetic Alterations
Juvenile polyposis syndrome	SMAD4, BMPR1A, PTEN	Deletion, insertion, missense & nonsense mutation
Peutz-Jeghers syndrome	STK11/LKB1	Deletion, insertion, missense, splicing mutations
Hereditary diffuse gastric cancer	CDH1	Frame shift & missense mutations
Familial adenomatous polyposis	APC	Frame shift & missense & nonsense mutations, deletions, duplication
Lynch Syndrome (HNPPC)	MLH1, MSH2, MSH6, PMS2, MSH3	Deletion & Duplication

E Pernicious anemia

In 1950 it was recognised as a important risk factor for gastric carcinoma[67]. And its considered to be a risk factor for non-cardia gastric cancer.[68]

F Polyp

Gastric polyps may be sporadic or associated with polyposis syndromes. E.g. FAP, PJS, juvenile polyposis, and cowden's disease[67]. Adenomatous polyp is well known recognised entity which may progress into malignancy. The risk (2-1.5%) of developing gastric

cancer varies according to the type ,size of polyp and grade of dysplasia[68].

G Epstein- barr virus

It is a well known causative agent for mononucleosis and also which has been related to burkitt's lymphoma, nasopharyngeal carcinoma and gastric cancer[69]

H. Occupations

A few number of occupations has been recognised as a risk factor for gastric carcinoma, which includes mining, fishing, timbre and farming as well as found in asbestos and rubber workers.

Other factors which are found to be associate with gastric cancer includes hypertrophic gastropathy, Metenier's disease, low socioeconomic status, chronic atrophic gastritis and obesity[33,70]

CLINICAL MANIFESTATIONS

Gastric adenocarcinoma lacks specific symptoms early in the course of disease, often ignore early vague epigastric discomfort and indigestion which are often mistake for gastritis. The epigastric discomfort similar to pain caused by benign ulcer. Typically this pain is constant, non radiating and cannot relieved by food ingestion. Locally advanced disease or metastatic disease produces physical signs during late in the course of the disease. Very large advanced tumours

often obstructs the lumen, which impairs distention of the stomach and produces symptoms like nausea, vomiting, fullness of stomach[33]. Clinically significant GI bleeding is rare, but as many as 15% of patients may develop hematemesis and anemia. Patients may present with apalpable abdominal mass, cachexia, enlarged palpable supraclavicular nodes (Virchow's) or periumbilical (Sister Mary Joseph's)[33,71] lymph node, peritoneal seeding palpable by rectal examination (Blummer's shelf)[3], or a palpable ovarian mass (Krukenberg'stumor) and may develop hepatomegaly and ascites and edema secondary to metastasis.

MACROSCOPIC CLASSIFICATION OF GASTRIC CANCER

(i) Early gastric cancer

Early gastric cancer is classified macroscopically into three types based upon the gross appearance of the lesion found on the mucosal surface (Figure 9). The endoscopic or macroscopic classification of gastric carcinoma recognised by Japanese Gastric Cancer Association. Now it has been widely accepted, which was approved in 2002 at an international workshop in Paris[57].

TypeI - Protruding type - These are polypoidal tumour more than 2.5mm in size, which projects above the level of mucosa.

Type II – Superficial type– This type subclassified into three groups:

Type IIa - Elevated lesion , equal or less than 2.5mm in size, the thickness of the mucosa is twice the thickness of normal mucosa.

Type IIb- Flat lesion, less than 5mm in size. Very difficult to diagnose through endoscopically.

Type IIc- Depressed lesion. The surface of the tumour is slightly depressed than the surrounding normal mucosa

Type III – Excavated type – This type shows prominent depression with ulceration.

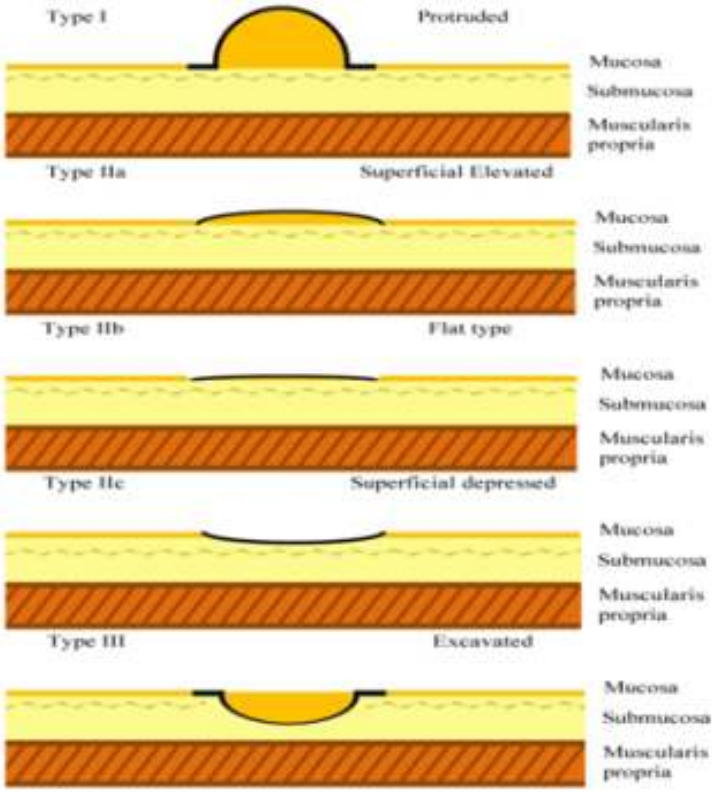


Figure 9: Gross classification of early gastric cancer

Approximately 80% of early gastric cancer are Type II lesions while diagnosed, and type IIc lesion is most commonest macroscopic type.

(ii) Advanced gastric cancer(AGC)

Borrmann classification is the widely used macroscopic classification for AGC. (Figure 10)

Type I – Polypoid- these are well circumscribed polypoidal tumours

Type II –Fungating- fungating tumours with central ulceration or infiltration.

Type III – Ulcerative –ulcerated tumours with infiltrative or heaped up margins.

Type IV – Diffusely infiltrative tumours.

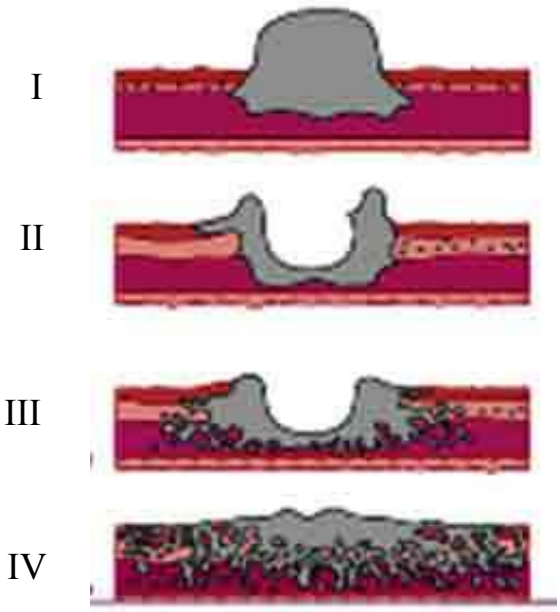


Figure 10. Borrmann classification of gross types of advanced gastric cancer

According to borrmann classification, type II tumours are the most commonest type in advanced stage, which are frequently seen in lesser curvature of the antrum. Type I & Type III tumours are usually found in greater curvature of the stomach. Infiltrative cancers can spread superficially in the mucosa and submucosa via lymphatics producing plaque-like lesions. It is usually accompanied by thickness of the entire stomach wall producing the so-called linitisplastica or leather bottle stomach.

CLASSIFICATIONS OF GASTRIC CARCINOMA:

Based on the histological appearance of the tumour, several classification systems has been proposed.

Ming classification

It classifies gastric adenocarcinoma into expanding and infiltrative types[72](Figure 11a,11b). The expanding type comprises 67% of gastric adenocarcinoma. It corresponds to the Lauren's intestinal type of carcinoma. It is characterised by aggregates of cells which is circumscribed and well delineated and surrounding tissue compressed aside.

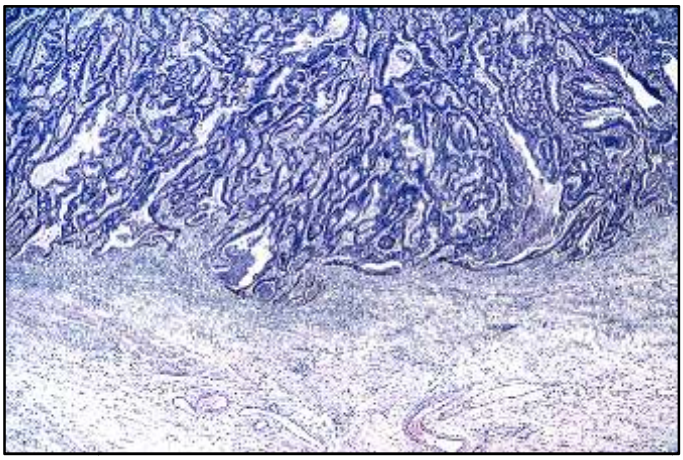


Figure 11a Expanding type of gastric carcinoma.

The infiltrative type of gastric carcinoma corresponds to Lauren's diffuse - type of carcinoma. It is characterised by wide infiltration of isolated, individual tumour cells. Expanding type tumours have better prognosis than infiltrative type of adenocarcinoma.

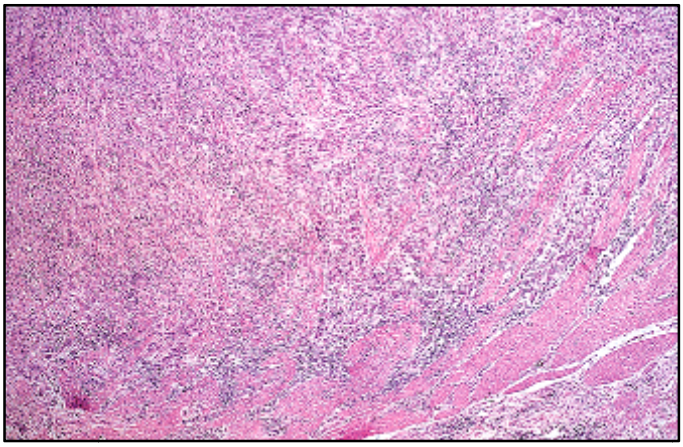


Figure 11b .Infiltrative type of gastric carcinoma

Goseki classification

Goseki proposed a classification based on the amount of mucus production and degree of tubular differentiation. This system provides

more accurate prognosis for advanced gastric cancer when it used with the tumour-node metastasis (TNM) system.

Carneiro classification

Carneiro et al proposed a much more simpler system in which the tumours are divided into glandular, isolated cell carcinomas, solid variety and a mixed type that consists of a mixture of glandular and isolated cell types[73].

Histologic classifications

Lauren's[74] has proposed two tier classification system based on their gland forming tendencies. The diffuse and intestinal type which corresponds to the undifferentiated and differentiated types respectively, according to Nakamura classification system.[75]

Mixed carcinoma consists of almost equal amount of diffuse and intestinal components. Indeterminate category consists of too undifferentiated carcinomas that cannot fit into either category[74]. Approximately the frequencies are 32% -diffuse type, 54%- intestinal, 15% of indeterminate type.

Intestinal carcinomas

These tumours usually have glandular pattern and they are thought to arise from metaplastic epithelium (Figure 12). The degree of differentiation inversely correlates with size of tumour. In well

differentiated tumours, most of the cells are mucin secreting or columnar in nature. Occasionally it shows complete intestinal metaplasia. The amount of mucin production is highly variable. And the stroma of the tumour is heavily infiltrated by histiocytes or neutrophils.

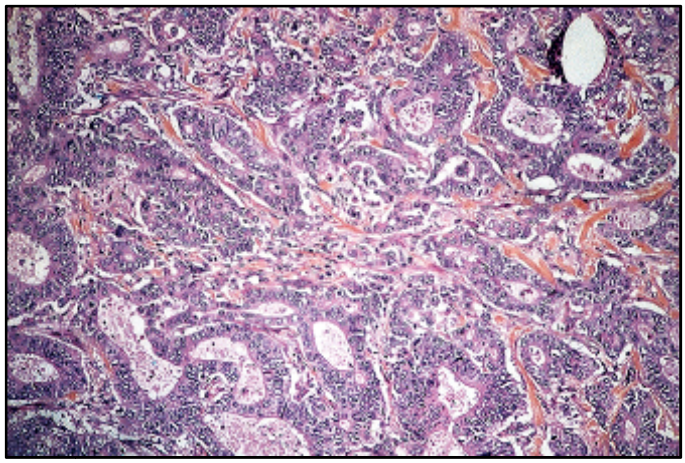


Figure 12. Lauren's & WHO intestinal type of gastric adenocarcinoma

Diffuse carcinomas

It usually occurs in young persons. These diffuse adenocarcinomas are classically known as linitis plastic and currently known as signet ring carcinoma. Sections from the gastric wall shows submucosal fibrosis and hypertrophic muscular wall which are evidenced by comb like appearance. Histologically it shows poorly cohesive malignant cells diffusely infiltrating the gastric wall with extensive fibrosis and inflammation. The cells usually grows individually and glandular formation are rare (Figure13). Intra cytoplasmic mucin secretion resulting in signet ring cell appearance.

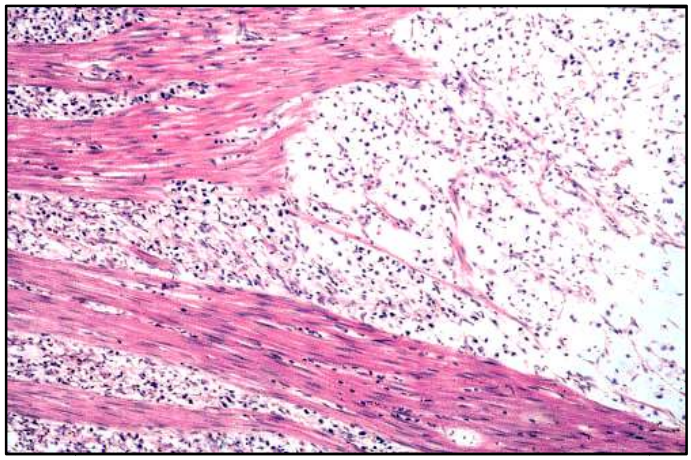


Figure 13 diffuse type of gastric carcinoma

The intestinal type of gastric adenocarcinoma has been considered to originate from the background of intestinal metaplasia and diffuse type of gastric adenocarcinoma originated from normal gastric mucosa. Both tumours follows different genetic pathways in tumorigenesis.[77,78]However, phenotypic cell markers of both types are extensively expressed in gastric adenocarcinomas, regardless of their microscopic type [79-82].

Phenotypic classification

Phenotypic classification of gastric adenocarcinoma based on the type of mucin secretion, which can be detected by using gastric and intestinal markers histochemically or immunohistochemically. Mucins are glycosylated glycoproteins secreted by gastro intestinal epithelial cells.[83-85]The secretary product of intestinal type of gastric carcinoma is an acid muco substance which can be easily detected by mayer mucicarmine stains. The mucin secreted by the diffuse type of gastric

adenocarcinoma are usually present within the cytoplasm of the signet ring cell carcinoma. Mucins are exclusively referred as MUC indicated with number, which exhibiting the order in which the mucin was derived. There is existence of correlation between the type of mucin secretion and tumour location. At the immune histochemical level the main mucin types expressed are MUC5AC for diffuse carcinoma, MUC1 for intestinal type carcinoma, MUC2 for the mucinous carcinoma and MUC5B for unclassified type of gastric adenocarcinoma.

Secretory mucins and transmembrane mucins are the two types of mucins described. Secretary mucins which act as a defense barriers for surface of gatric epithelium. Transmembrane type of mucins which act as a ligands in cell signalling pathways[85]. Mucin plays a important role in normal physiological functions and also in pathological changes in tumour metastasis.

There are two types of mucus secreting cells in gastric mucosa. They are glandular mucous cells and surface mucous cells[83]. The expression of mucin in gastric adenocarcinoma is heterogeneous. It consist of gastric phenotypic markers like MUC5AC, MUC1, MUC6 and intestinal phenotypic marker MUC2[84,85]. According to this

phenotypic markers expression, gastric adenocarcinomas are classified into following four differentiated types[79].

- ***G type tumours*** - It shows positivity for gastric phenotypic marker, not for intestinal marker.
- ***I type tumours***- This tumour expresses only intestinal phenotypic marker, never express gastric marker.
- ***GI (combined) type*** - It shows positive results for both intestinal and gastric markers.
- ***UC (unclassified type)***- It does not show positivity for any of the phenotypic marker.

World Health Organization classification

In 2010 World health organization recognises four important histologic types of gastric adenocarcinoma. They are tubular carcinoma, papillary adenocarcinoma, mucinous carcinoma and poorly cohesive carcinoma which includes signet cell carcinoma. Several other rare variants are included under WHO classification [41](Table 4). The classification is based on the principal or dominant histological pattern, which may be associated with other types of the carcinoma .

WHO (2010) Classification of gastric carcinoma[68] (Table4)
Papillary adenocarcinoma
Tubular adenocarcinoma
Mucinous adenocarcinoma
Signet-ring cell carcinoma
And other poorly cohesive carcinoma
Mixed carcinoma
Adenosquamous carcinoma
Squamous cell carcinoma
Hepatoid adenocarcinoma
Carcinoma with lymphoid stroma
Choriocarcinoma
Carcinosarcoma
Parietal cell carcinoma
Malignant rhabdoid tumour
Mucoepidermoid carcinoma
Paneth cell carcinoma
Undifferentiated carcinoma
Mixed adeno-neuroendocrine carcinoma

Continued....

Endodermal sinus tumour
Embryonal carcinoma
Pure gastric yolk sac tumour
Oncocytic adenocarcinoma

Tubular adenocarcinomas

Tubular adenocarcinoma is the commonest histological entity of EGC. It usually forms fungating or polypoidal mass and histological picture shows predominantly composed of neoplastic tubules with varying diameter often shows irregular branching and anastomosis which embedded in the fibrous stroma and conspicuous desmoplasia (Figure 14)Individual tumour cells are columnar, cuboidal, or flattened by intraluminal mucin. The degree of atypia varies from high to low grade[87,88].

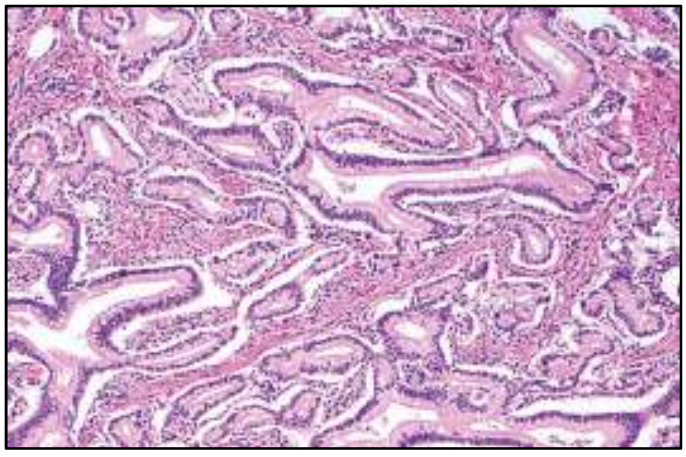


Figure14. Neoplastic, branched tubules in tubular adenocarcinoma

A poorly differentiated type is otherwise known as solid carcinoma. Another variant known as oncocytic variant has been recognised .A poorly differentiated variant is sometimes called solid carcinoma.

Papillary adenocarcinomas

It is an another important histological variant found in early gastric cancer. Usually common in older individuals, and frequently shows liver and nodal metastasis. These are well-differentiated and well demarcated tumours with pointed or blunt elongated finger-like processes which are lined by cuboidal cells with central fibrovascular cores and admixed with acute and chronic inflammatory cells (Figure15). Some of the tumour shows papillo tubular differentiation which often coexist with micro papillary architecture. It may be associated with cellular atypia , nuclear pleomorphism with varying mitotic index.

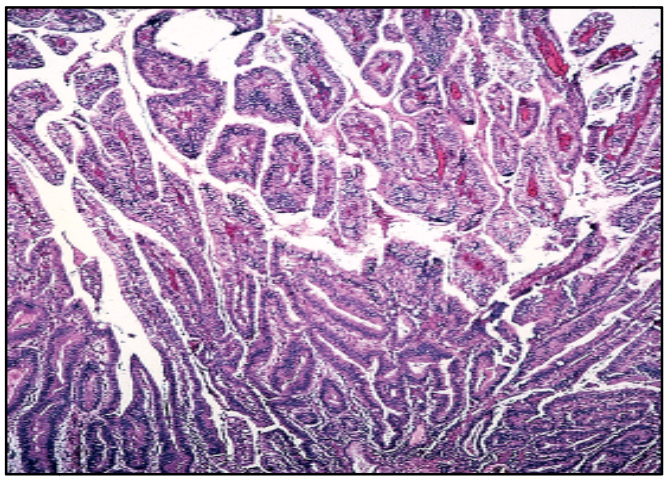


Figure 15. Papillary projections lined by neoplastic cells.

Mucinous adenocarcinomas

It is characterized by prominent glandular structure with large amounts of extracellular mucin in atleast 50% of the tumour cells. It represents 10 % the of gastric cancer. It has two important growth patterns. Formation of glands which are lined by mucus-secreting columnar cells (well differentiated type).

- ❖ Tumour cell arranged as a disaggregated irregular ribbons which appears to be float in the lakes of mucin (poorly differentiated type) (Figure 16).
- ❖ Mucin also be present in inter-glandular stromal areas which can be associate with scattered signet-ring cells. Grading of tumours is unreliable if the tumours contains only few cells.

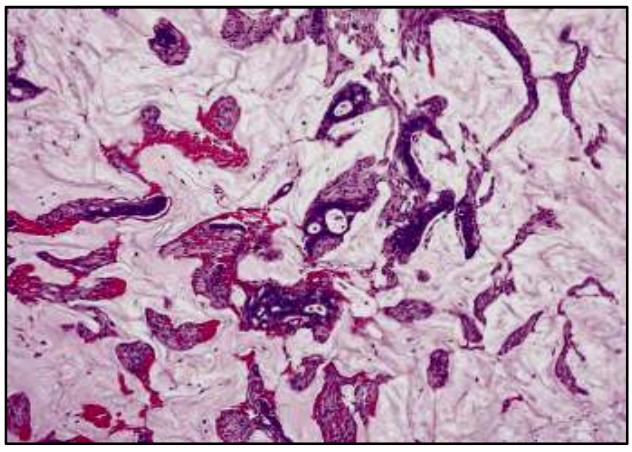


Figure 16 Clusters of neoplastic cells float in lake of mucin

Signet-ring cell carcinomas

By definition it comprises isolated single cells or tight cluster of cells with an eccentrically compressed and displaced nuclei having

intra-cytoplasmic mucin which accounts for more than 50% of the tumour (Figure 17). These tumours are more common in younger patients and in the distal stomach.

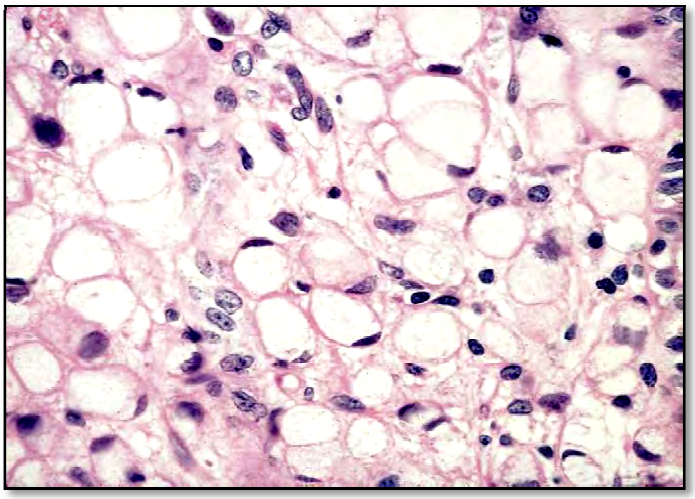


Figure 17 prominent intracellular mucin with eccentrically pushed nucleus

Five morphological pattern of tumour cells have been described.

1. Nuclei which push against cell membranes and forms classic signet ring cell appearance due to clear and expanded cytoplasm. It usually contain acid mucin which stains for Alcian blue at the pH of 2.5
2. some of the signet cells resemble signet ring cell which shows centrally placed nuclei with little mitosis.
3. signet cell contains neutral mucin instead of acid mucin with deeply eosinophilic cytoplasm and prominent distinct cytoplasmic granules.
4. signet cells which is small in size with little mucin

5. anaplastic cells with or without mucin

These signet cell types were mingled with each other and constitutes varying tumour proportions. It may also form lacy delicate glandular pattern. Signet cell carcinomas are usually infiltrative in nature with prominent desmoplasia. It has great tendency to invade duodenum through the sub mucosal and sub serosal lymphatic channels, special attention is needed to those routes when frozen sections were requested to evaluate marginal status.

Special stains which includes PAS, alcian blue, mucicarmine or cytokeratin immunostaining are required to identify the sparsely distributed malignant cells in the stroma. Cytokeratin immunostains which is superior than mucin stain to detect neoplastic cells.

Several other conditions can mimic signet-ring cell carcinoma which includes signet-ring lymphoma, xanthomas, laminapropria muciphages. Benign pseudo-signet ring cell is the important differential diagnosis for malignant signet ring cells. Even though this pseudo-signet ring cells demonstrates cytological atypia and mitoses it doesn't show invasion. Reticulin stain can highlight the pseudo-signet ring cells which is limited to basement membrane and the acinar architecture is usually intact[89].

Micropapillary carcinoma (MPC)

Micropapillary carcinoma is a newly diagnosed aggressive rare histologic variant characterized by tiny papillary clusters of malignant cells which are surrounded by clear spaces with indistinct fibrovascular core(Figure18). These micro papillary features are usually prominent in advancing edge of tumour, with high incidence of nodal metastasis via endolymphatic tumour emboli. The prognosis of the micropapillary carcinoma, not significantly different from conventional gastric carcinoma [107]. Conservative treatment not advised for micro papillary carcinoma because of higher incidence of lymphatic invasion[109]

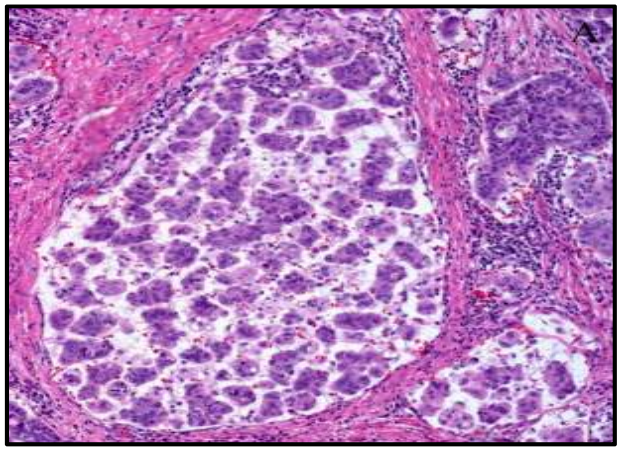


Figure 18 Micro papillary carcinoma lined by a space without lining epithelium.

Other variants

World Health Organisation also recognised other rare histologic variants, which are described below.

Medullary carcinoma

Gastric carcinoma with lymphoid stroma is also known as medullary carcinoma. It is one of the uncommon variant with less aggressive clinical course. It usually occurs in the antral/ body of the stomach. Males are more prone to this type of carcinoma.

Histological picture shows well demarcated tumour composed of polygonal tumour cells which arranged in irregular nests or sheets with a prominent stromal lymphoid infiltrate without desmoplasia (Figure 19a).

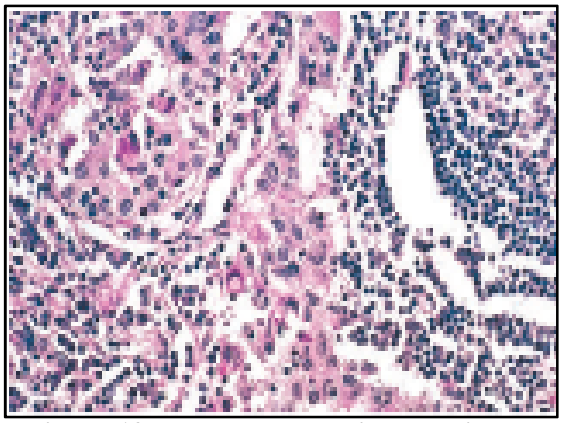


Figure 19a medullary carcinoma with lymphoid stroma

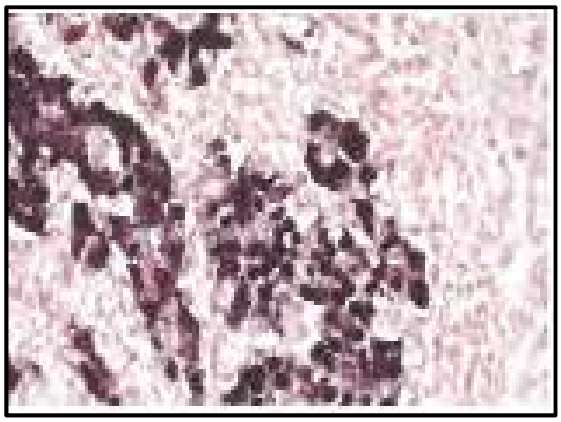


Figure 19b Epstein–Barr virus DNA within tumour cells but not in lymphoid stroma. (*In situ* hybridization)

Epstein-Barr virus (EBV) infection can be demonstrated in more than 80% of medullary carcinoma[89,90](Figure 19b). This finding has increased hope for tumour cell targeting, by using a proteasome inhibitor which induces EBV kinase activity which in turn helps to kill the targeted by other agents[91]. High microsatellite instability was noted in

another group of GCLS resulting from defective function of hMLH1 or hMSH2[92,93-95].

Adenosquamous carcinoma

It accounts for less than 1% of cases. This lesion usually demonstrates adeno and squamous cell carcinoma pattern (Figure 20) . The collision tumour composed of two histologically distinct tumour pattern. Tumours often shows foci of squamous metaplasia are known as adenoacanthoma.

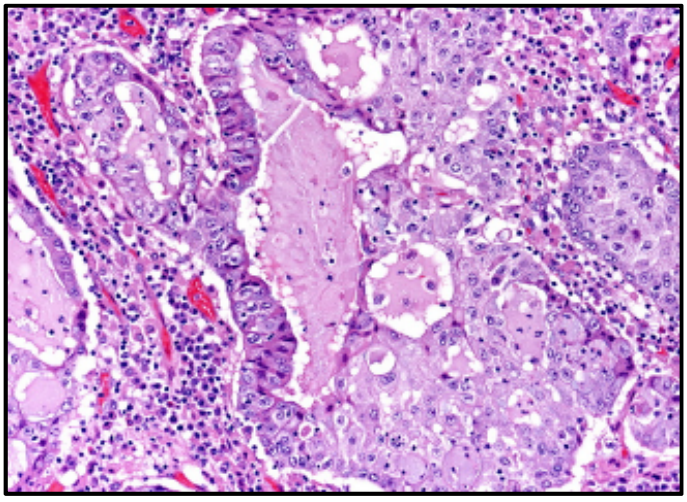


Figure 20 Focus of squamous cells with mucinous adenocarcinoma

Squamous cell carcinoma (SCC)

It is a very unusual in stomach, usually resembles SCC originates elsewhere in body. Histological picture shows malignant squamous cells with moderate pleomorphism (Figure 21).

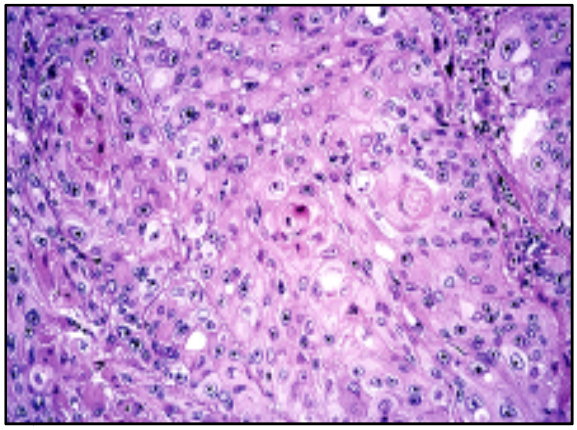


Figure 21 Gastric squamous cell carcinoma

Undifferentiated carcinoma

Tumours lack differentiation, but exhibit cytokeratin expression are included under undifferentiated carcinoma according to WHO. Further analysis by using histochemicalmethods needed for their separation into other tumour types.

Hepatoid and α-Fetoprotein–producing carcinomas

Its another rare entity, there are foci of neoplastic tumour cells which are large polygonal cells resembles hepatocellular carcinoma, admixed with the intestinal type of gastric adenocarcinoma (Figure 22) . These are bulky tumour with polypoidal nature associated with ulceration, necrosis and haemorrhage. A specific feature of these tumours are extensive infiltration of venules, which causes high incidence of hepatic metastasis and worse prognosis than conventional gastric carcinoma[98,99,40].Some published studies has been explained the incidence of this variant ranges from 1.3% to 15% of all gastric

carcinoma.It is unusual below the age of 50. The most common location for this tumour is antrum.The diagnostic interpretation is very clear when evaluating the primary tumour, but its very difficult when evaluating secondary liver metastases. Serum AFPis usually elevated in affected patients and immunohistochemistry can demonstrates within the malignant cell[40].

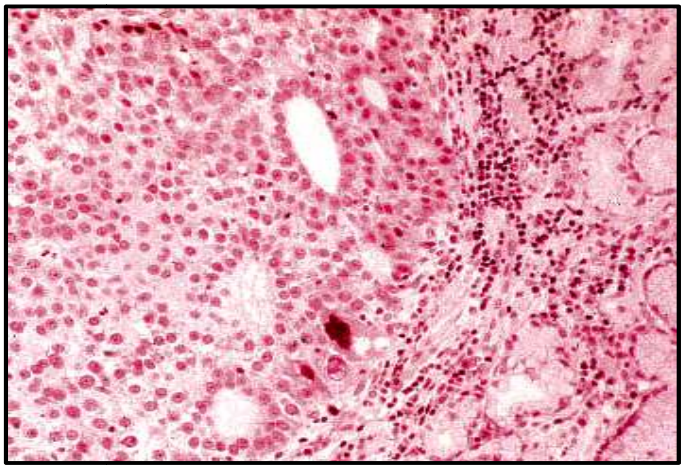


Figure 22Hepatoid gastric carcinoma showing uniform tumour cells (left) arising in gastric mucosa (right).

Choriocarcinoma

Pure form of gastric choriocarcinomas are very rare. Most of the cases shows combination of trophoblastic elements within variably differentiated adenocarcinoma (Figure 23). Prominent necrosis and hemorrhage are usually evidenced . Immunohistochemistry can demonstrate human chorionic gonadotropin, and elevated HCG levels in the serum can be used as a prognostic marker. Recently accepted pathogenic explanation is

that these neoplasm represents choriocarcinomatous differentiation of the conventional adenocarcinoma.

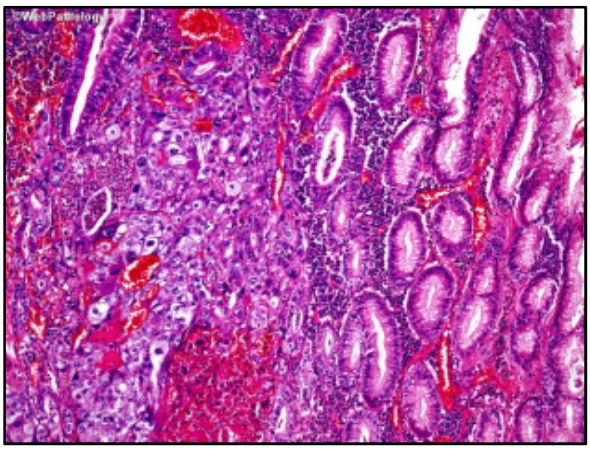


Figure 23 Hemorrhagic neoplasm with many multinucleated syncytiotrophoblasts

Carcinosarcoma

Carcinosarcomas is very rare in the stomach . Tumours composed of both adenocarcinomatous and sarcomatous components. Sarcomatous elements comprises uncommitted cells or differentiating cells like chondrosarcoma, leiomyosarcoma,osteosarcoma, rhabdomyosarcoma.

Small cell carcinoma

It is a rare tumour only 100 cases have been reported till now. These tumours are usually diagnosed at advanced stage , so the prognosis of this tumour is quite poor. Most patients usually die within 1 year of diagnosis. The tumours resembles their pulmonary counterpart, showing neoplastic cells arranged in sheet like configuration with infiltrative growth , rosette formations, peripheral palisading of nuclei, inconspicuous nucleoli (Figure 24) .

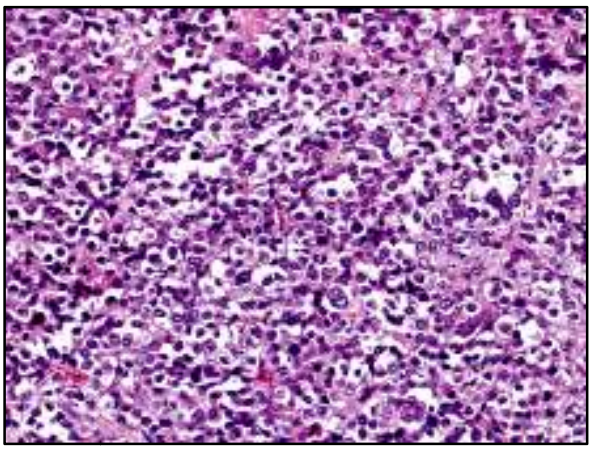


Figure 24 shows solid sheets and cords of small "blue" cells.

IHC study shows positivity for neuron-specific enolase and chromogranin and negativity for carcino embryonic antigen. Characteristic neuro secretory granules can be demonstrated by electron microscopy.

Parietal cell carcinoma andoncocytic carcinoma

Parietal cell carcinoma is an exceedingly rare tumour. These neoplasm has an expanding growth pattern which composed of polygonal arranged in solid sheets with abundant, granular, eosinophilic cytoplasm that can be stained by phosphotungstic acid–hematoxylin (Figure 25). It has good prognosis than the conventional gastric adenocarcinomas.Table 5 summarises uncommon variants and special studies which used for their diagnosis

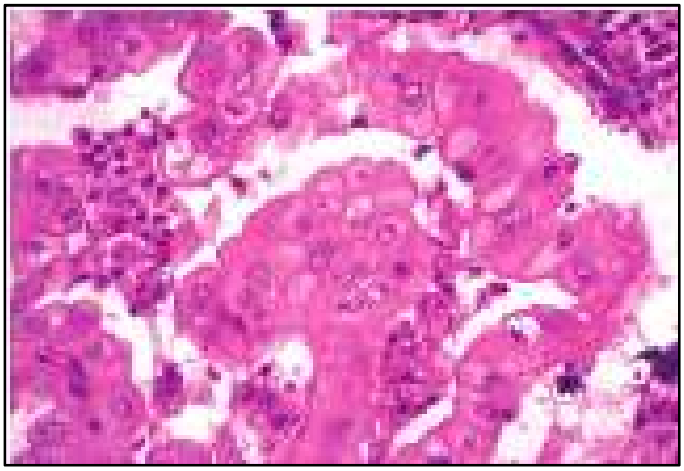


Figure 25 polygonal cells with abundant granular cytoplasm

Table 5 Pathological characteristics of rare variants

Histological Type	Histology	Special studies
Hepatoid adenocarcinoma	Large polygonal cells, Bile & PAS+ve intracytoplasmic eosinophilic globules	AFP (+) – insitu & serum
Medullary carcinoma with lymphoid serum	Poorly developed tubular structure with prominent stroma	>80% tumours associated with EBV infection
AdenoSquamous carcinoma	>25% of squamous component	-
Squamous cell carcinoma	Moderately differentiated to poorly differentiated	-
Gastric small cell carcinoma	Sheet like growth with rosette like arrangement	Chromogranin A and NSE +ve, CEA -ve

Gastricmucoepidermoid and Panethcell carcinomas

Mucoepidermoid carcinoma shows mixed population of mucus producing cells and squamous epithelium.Paneth cell carcinomas is

characterized by neoplasm with Paneth cell differentiation. Immunohistochemistry reveals characteristic cytoplasmic granules that showing positivity for lysozyme.

Malignant Rhabdoid Tumour

It accounts for nearly 0.1% to 0.2% of all gastric cancers. Histological picture shows poorly cohesive neoplastic polygonal cells with clear or acidophilic cytoplasm with large centrally placed nuclei and conspicuous nucleoli (Figure 26). These rhabdoid cells expresses vimentin, cytokeratin, EMA, focal NSE, but shows negativity for CEA.

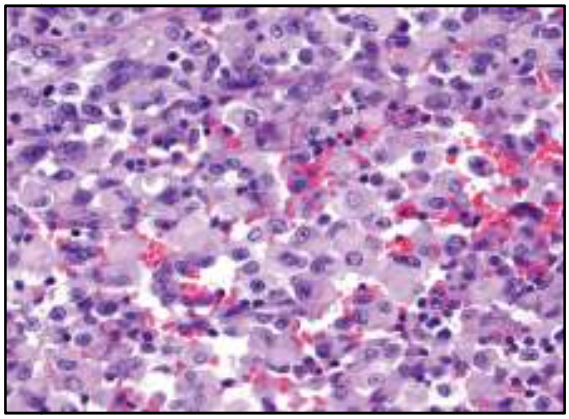


Figure 26 Largediscohesive cells with characteristic eosinophilic cytoplasm and prominent nucleoli.

Early gastric cancer

EGC is a carcinoma in which neoplastic tumour cells are limited upto mucosa or submucosa, irrespective of the nodal involvement (Figure27). Majority of EGC are asymptomatic and usually occur in elderly individuals. Rate of growth of EGC is usually slow. Follow-up

study of dysplastic lesions usually demonstrates the high prevalence of EGC. Histological picture shows either pure form or mixed form. Grossly raised lesions with papillary or nodular patterns often shows well or moderately differentiated intestinal carcinoma. Flat lesions usually shows poorly differentiation . Grossly ulcerated tumours consist of either diffuse or intestinal carcinoma.

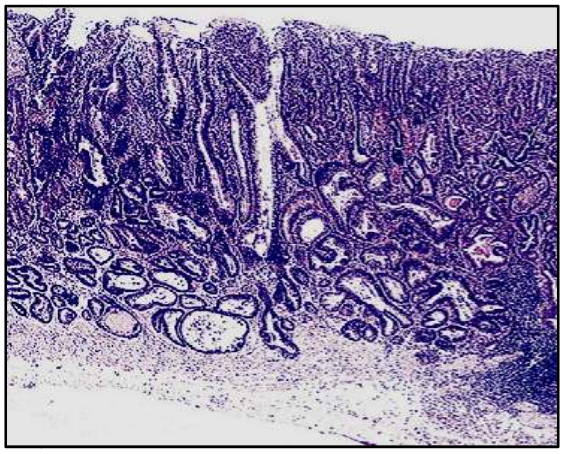


Figure 27 Early Gastric cancer showing mucosal involvement

Adenocarcinoma which is limited to mucosa has been classified into 1) small mucosal (less than 4cm) 2) superficial (more than 4cm)[100]. If they shows focal infiltration into sub-mucosa, it has been classified as small mucosal SM and superficial SM.

If there is extensive sub mucosal infiltration than above-mentioned variants, it is known as penetrating variant. It is sub divided into PenA and PenB . PenA shows only pushing margin, and PenB usually penetrates muscularis mucosae.[100]

Stromal reactions

These are the usual stromal responses to the neoplastic cells. They are desmoplastic stroma, tumour lymphocytic infiltrates, eosinophilic infiltration of stroma and granulomatous response. These granulomatous reaction is evidenced by presence of single or confluent sarcoid-like granuloma areas admixed with intense infiltration of mononuclear cell.

Grading

- ***Well differentiated:*** Tumour composed of well-formed glands, often resembles metaplastic intestinal epithelium.
- ***Moderately differentiated:*** Tumour composed of glandular elements , morphologically intermediate between well to poorly differentiated carcinoma.
- ***Poorly differentiated:*** Tumour shows irregular glandular elements that are difficult to recognize , which arranged in single cells or small clusters with acinar structures.

Tubular carcinoma may also be graded as low-grade or high-grade (poorly differentiated).

SPREAD OF GASTRIC CARCINOMA

Direct spread

In resected specimens the majority of gastric cancer tumours have extended into sub serosa and serosa and its extent is greater in infiltrative tumours. Spread to the adjacent organs depends on the location of the primary tumour growth. Lower end of oesophagus can be infiltrated by the gastric carcinoma which arise from the proximal portion of the stomach and tumours arise from the distal part of the stomach shows microscopic extension into the duodenum[101]. Local extension can also occur in omentum, pancreas, transverse colon and spleen. The diffuse type of carcinoma shows wider dissemination than the intestinal type of carcinoma. Widespread direct spread is especially common with signet ring carcinomas.

Lymphatic spread

Lymph node metastases can be found in 70% of surgical resections and in 90% of gastric carcinomas at autopsy[102]. The nodal metastasis of the tumour is declining now, because more lesions are diagnosed at early stage. Depth of tumour invasion directly proportional to the incidence of the lymph node metastasis, irrespective of the microscopic type of primary tumor[210]. The tumour can metastasise to perigastric, peri aortic

and celiac nodes via submucosal and mucosal lymphatic plexus[104]. hepatoduodenal nodal spread is evidenced in distal gastric tumours[105].

Haematogenous spread

Vascular invasion can occur even in the absence of nodal metastasis. The most common location for distal metastasis are liver which is followed by peritoneum, lung and ovaries. The diffuse type of gastric carcinoma can spread through peritoneal seeding whereas intestinal type of gastric carcinoma usually spread via vascular invasion. Intestinal type tumours may involves rare sites such as spleen, meninges, kidney and uterus.[106,107]

And gastric carcinoma in younger age group more prone for peritoneal metastasis. If the liver metastasis shows diffuse pattern or peritoneal seeding shows intestinal pattern of growth , the primary carcinoma often shows mixed pattern.

Transperitoneal spread

Secondary metastatic deposits from the primary gastric adenocarcinoma are common in peritoneum, omentum and mesentry. Metastatic ovarian deposits from primary gastric adenocarcinoma known as krukenberg's tumour, which is commonly associated with diffuse type.

MOLECULAR PATHOGENESIS

Gastric cancer is a heterogeneous disease, and gastric carcinogenesis is a multistep process involving multiple genetic and epigenetic alterations associates with environment conditions. Infections usually initiates chronic inflammation which initiates sequence of reactive changes, which prone to development of carcinoma. The molecular pathogenesis of gastric carcinoma is well exemplified in the pathogenesis of colonic cancer and also noted in intestinal type of stomach cancer[108]. But it is well demonstrated in the precancerous cascade[109].The established sequence of molecular events that occur during the development of gastric carcinoma is shown (Figure 28)

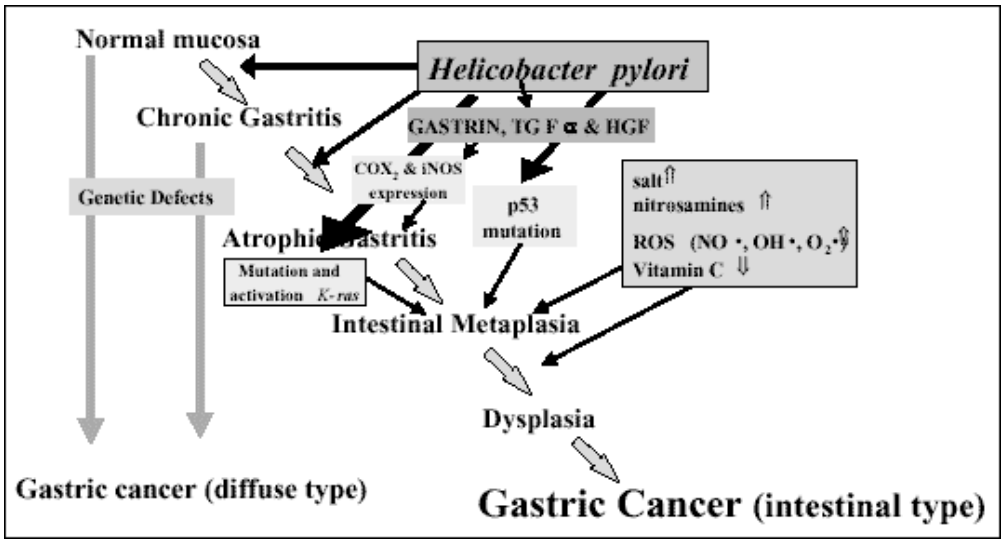


Figure 28 Multi step molecular pathogenesis of Gastric cancer

The most common changes, whether genetic or epigenetic, that occur during the development from inflammation to carcinoma are clustered at genes involved in cellular regulatory pathways.

The four important pathways narrated here are interrelated with each other and they are not considered to be independent, as these components interact with each other and control the cellular growth and proliferation.

Oncogenes and Tumour suppressor gene

1. The p53 is the most commonly mutated tumour suppressor gene which controls the gene expression in DNA damage, cell cycle arrest to permit repairing of DNA , or cell apoptosis. Under physiological or unstressed situations it combines with MDM2 which targets rapid degradation of p53. Inhibition of this pathways leads to functional activation of p53, and it undergoes several post –transcriptional modifications which induces cell cycle and apoptosis arrest via p21. TP53 mutation or LOH has been reported around 60% of all gastric cancers[108]. Mutation of p53 can occurs in earlier part of tumoriogenesis and also increases during the progression of cancer. But it is rarely found in early gastric lesions[110]

2. G1 phase of cell cycle regulated by RB1 (retinoblastoma) pathway genes and p16 genes. Decreased expression of p16 found in 47% of gastric cancers.[111]
3. The TGF-"R2 (transforming growth factor – type 2 receptor ") gene is particularly susceptible to microsatellite instability, which leads to loss of growth inhibition, which considered to be a most important feature of gastric carcinoma[112].
4. The APC (adenomatosis polyposis coli) " has a higher frequency of mutation in 20 – 70% of gastric adenoma and flat dysplasia. Their importance is less established in the development of gastric carcinoma when compared to colorectal cancer.

Micro satellite instability (MSI)

Dysfunction and inactivation of the DNA mismatch repair system is responsible for MSI. The estimated frequency of Micro satellite instability to be around 30% in gastric adenocarcinoma. LOH of the APC , cytosine – adenine repeat instability have been documented in well differentiated carcinoma.[113]

Microribonucleic acids (miRNAs)

miRNAs can act as both oncogenes and tumour suppressor genes, which regulates multiple biological processes. They are usually found within the regions of LOH, amplification, genomic regions

which is associated with cancer. Dysregulation of miRNAs plays an important role in gastric carcinogenesis. Recent Studies have shown microRNAs that function as tumour suppressors (miRs-101 , miRs-181 , miRs-486, miRs-449) were down regulated, whereas miRNAs that function as a oncogenes (miRs -21 , miRs-17 , miRs 106a) were up regulated in gastric carcinoma.[117\4] Intestinal (27%) type of gastric carcinoma can be associated with β- catenin mutations[115].

E–cadherin and the Wnt system

Wnt signal transduction pathway is a central mechanism for regulating gene expression. β-catenin normally binds to the intracellular domain of E-cadherin (Figure 29).In the absence of Wnt ligand , the destruction complex (GSK3β, APC, Axin) creates a hyperphosphorylated β-catenin, which is a target for Ubiquitination and degradation by the proteasome. Binding of Wnt ligand to a Frizzled/LRP-5/6 receptor complex (right panel) leads to stabilization of hypophosphorylated β-catenin. Then it interacts with TCF/LEF proteins in the nucleus to activate transcription, where it can exert oncogenic role. The whole Wnt pathway becomes independent of E-cadherin expression, if there is any alteration in Wnt pathway[103]. This accumulation of β-catenin can be explained by mutations in APC gene (Adenomatous Polyposis Coli) or in β-catenin gene itself. E-cadherin binds to the actin cytoskeleton via a series of catenin proteins. Loss

of E-cadherin complexed to the cell membrane is associated with an increase in the share intracytoplasmic and nuclear of β-catenin[106].A mutation of the APC gene appears be found in approximately 30% of intestinal adenocarcinomas[107]. To further support the role of Wnt, has observed that patients with germline mutations of APC present a risk of developing gastric cancer 10 times higher than that of the normal population.

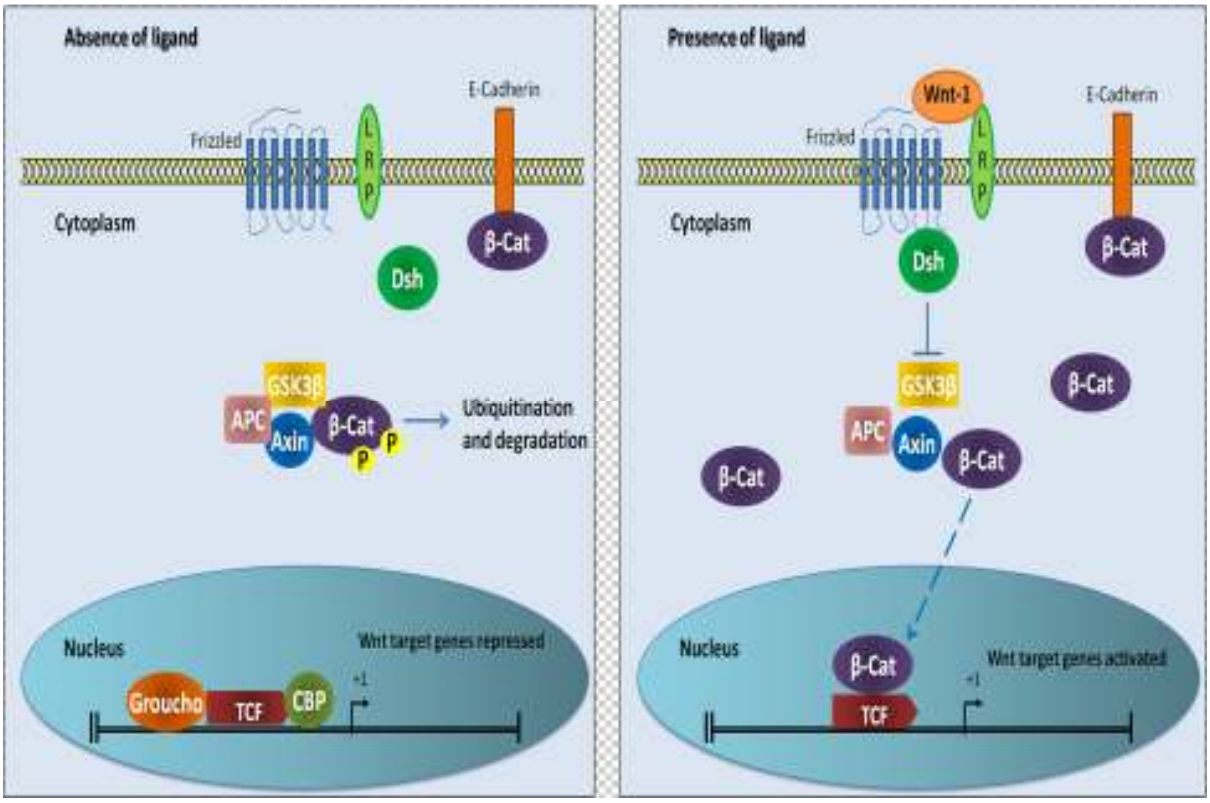


Figure 29.The Wnt signaling pathway.

Chromosomal instability(CIN)

Gastric carcinoma which is sporadic in nature has been associated with CIN, Multiple factors have been contributed to CIN in gastric cancer patients, which includes DNA damage response,

H.pylori infection, aberrations in chromosomal segregation, dietary nitrates[116]. It may manifest as loss or gain of part/whole of chromosomes. A higher frequency of LOH was found in p53, APC, Rb loci and nm23.

Epigenetic changes

DNA methylation, histone methylation and histone acetylation which has been recently documented epigenetic alterations leads to gene alterations.

(i)DNA methylation

Silencing of tumour suppressor genes may occur when it is associated with hypermethylation of CpG islands, which have been identified in gastric carcinoma that serve as a good prognostic indicators[117]. Mutations in E-cadherin (member of the APC pathway) have been recognised in 50%of diffuse-type of gastric cancer.[108]

(ii) Histone acetylation

Histone deacetylation is associated with transcriptional repression of multiple tumour suppressor genes by inactivating chromatin. Hypoacetylation of histones H4 & H3 in p21 promotor region is observed in more than 50% cases[118]. 70% of gastric carcinoma expresses reduced level of acetylated histones H4.

As discussed above, the components affected by mutation varies. Each patients with gastric carcinoma expresses different combinations of mutations, often in tumours with same histological type. Even same type of tumours react differently to treatment. Each cancer exhibits its own array of genetic alterations which leads to tumoriogenesis and it shows high variability. Although many tumour suppressor genes and oncogenes are discovered so far, there is still need for evaluation and detection of genes which involved in the cellular pathways .A wide genomic research is required to detect the novel methylation silenced genes in gastric cancer, which will help to understand molecular genetics of GC and to provide new treatment opportunities.

TREATMENT

1. Curative resection

Even in the absence of metastatic spread, aggressive surgical resection of gastric tumour is justified. Curative resection (R0) is defined as a complete resection of tumour with resected margin free of malignancy, both grossly and microscopically[119].R1 is known as resection with residual microscopic disease, and R2 is known as resection with gross residual disease. Survival rate is good in patients who underwent R0 resection for local disease. Several published studies

described that the curative surgical management determined by the surgeon is a most important, independent prognostic factor [120].

Gastrectomy procedures which includes total gastrectomy, subtotal gastrectomy and proximal total gastrectomy. Tumours located in the antrum an distal body can be resected by subtotal gastrectomy. Tumours originated from the cardia resected by proximal total gastrectomy.

2. Lymph node dissection

Involved lymph nodes also requires to be removed along with radical and curative resection. The incidence of nodal involvement which ranges from3% to 5% for tumours restricted to the mucosa, 16% to 25% for tumours confined to submucosa, and 80% to 90% in patients with stage III or stage IV disease. The extent of lymph node dissection (LND) is not well defined. There is also no convincing evidence that extended lymph node dissection, as advocated by some Japanese surgeons, significantly increases survival.[121]

The five-year survival rates of the extended LND is increased when compared to limited resections. A minimum of 15 lymph nodes should be sampled by the surgeon and reported by the pathologist for an adequate pathological staging.[122]

Gastric carcinoma responds little to radiation therapy andit is also relatively unresponsive to chemotherapy.[123,124] Recently, HER2/neu has been validated as a molecular target for this disease.[125]

3.Palliative treatment

The goal of palliative treatment is to relieve the pain with minimal morbidity. Surgical palliation usually done for advanced gastric tumours, which includes surgical resection, bypass alone or in addition with endoscopic and radiotherapy technique. Complete staging of tumour is necessary to select the proper method of palliation for individual patients.

STAGING OF GASTRIC CANCER :

The TNM staging system (Annexure III) is widely used in western countries. It is the best available predictor of prognosis and is recommended.

PROGNOSTIC FACTORS

Prognostic factor is defined as any variable pathological factor that provides useful information to assess the outcome of disease at the time of diagnosis. These pathological factors are TNM staging, macroscopic type of tumours, site of tumour origin, histological type, nodal involvement, vascular invasion, lymphatic invasion.[126]

Age and Gender

Gastric carcinoma is common among males than females(2:1) in elderly patients[127]. The gender difference is usually not seen in young individuals. Gastric cancer usually diagnosed around the age of 60 – 70 years. Increased incidence of gastric cancer is found in older individual. These gender differences usually not seen in younger patients and 10% of gastric cancer may occurs around the age of 40 years.[127-129] In younger patients with gastric cancer are more prone to diffuse type of carcinoma[130,131]. Some studies published that prognosis of gastric cancer does not seem to be affected by age, once the survival data is adjusted for TNM stage.[131,132]

Grade

Pathologic grading system classifies tumours into 3 categories. They are well differentiated, moderately differentiated or poorly differentiated. This degree of differentiation has been shown to correlate with the aggressive forms of malignancy.[133] Even though grade is usually reported in pathological reports, their significance in gastric carcinoma remains unclear. Several published studies failed to conclude pathological grade as an independent prognostic factor[134-136].

Size

Several retrospective studies revealed prognosis is associated with the size of the tumour.[136 -138]Even though it is one of the powerful predictor of prognosis, it doesn't seems to have a prognostic significance when compared with depth of penetration and nodal metastasis.[139]

Tumour Location

The location of the tumour has a several valuable implications in the management and prognosis of gastric cancer. Several studies has demonstrated gastric cancer originates from proximal stomach shows a distinct clinical entity and prognostic association.[137-141] Few studies failed to find association between tumour location and prognosis[142]. A recent study published that tumours located in proximal stomach have extensive gastric wall penetration, vascular invasion, lymph node metastasis, advance stage, with an overall poor survival rate when compared to distal tumors.[140] So that different surgical approach is required for proximal tumours because of its aggressive biological behaviour.

Lymphatic and Vascular Invasion

Recently, studies related to lymphatic and vascular invasion gained popularity for predicting tumour behaviour. Studies have shown

that nodal involvement is important predictor of survival, and the existence of tumour emboli , significantly raises the recurrence of tumour and death even after curative resection.[138,143] They are usually associated with decreased survival rates.

TNM Staging

TNM is the most significant prognostic factor. One of the features that it incorporates is the degree of infitration[144]. Tumours with increased depth of invasion have the greater chance of metastasis. This feature is related to the macroscopic appearance of the tumour. Tumour grows primarily within the gastric wall have a higher incidence of metastasis than the intraluminal neoplasms. Five year survival rates are 91% for stage Ia tumours, 64% for stage II tumours, 27% for stage IIIa tumours, and 0% for stage IIIb/IV tumours[144].

And Survival rate of patients with EGC invading mucosa but limited submucosa being 90-100%, compared with 60-80% for gastric tumours invading the muscularis propria, and 41-50% for tumours which is limited to the subserosa or serosa.[144,145]Survival rate for advanced gastric carcinoma is lower than 23% , and the prognosis of this advanced lesions remains poor.[145]

Lymph node metastasis

The depth of tumour invasion correlates with the presence nodal metastasis, and the presence of regional nodal metastasis decreases the five year survival rate of EGC from 90% to 70%. The lymph node status and the ratio of involved and uninvolved lymph nodes are the important markers of gastric carcinoma prognosis.[126] The Nodal ratio (N-ratio) has been recognised as an important independent prognostic factor, even where less than the fifteen lymph nodes have beenanalysed.[146] The five year survival rate is 44% for patients with 1-6 lymph nodal metastases, 30% for 7-15 nodal metastases, and 11% if more than 15 lymph nodes shows deposits. And five year survival rate is 83.4% for the cases showed zero N ratio, if the N ratio is 1 the survival rate could be 66.3% , 46 % for the cases showed N ratio 2 , and 19.0% survival rate for the N-ratio 3. Unfortunately, most of patients with advanced gastric carcinoma already presents with nodal metastases.

Histological classification

Diffuse type of gastric tumours and mucous-rich tumours may predict a poor prognosis[1]

Borrmann's classification

Borrmann has categorized the gross appearance of gastric tumours into four types.[147]

Borrmann type IV cancer usually shows an unfavourable prognosis.[155,156]

Other Factors

Multiple other factors have been associated with higher incidence of local recurrence and reduced survival. Tumour markers like p53, CD 34, E-cadherin, HER2/neu, CA 72–4, CEA have been considered as potential prognostic markers for predicting the behaviour of tumor[140, 150,151] The recognition of reduced expression of E-cadherin act as an indicator of worse prognosis.[152,153] The impact of additional molecular genetic alterations over the prognosis of gastric carcinoma is currently the field of interest[154,155]

Human epidermal growth factor 2(HER2/ neu)

TNM stage is the most important factor in determining the prognosis of gastric cancer. However, in patients with the same stage, prognosis could be various, so further research studies are needed to identify new prognostic factors.

HER-2 is a proto oncogene, which is located on chromosome 17q and encodes a transmembrane tyrosine kinase receptor protein that is a member of the epidermal growth factor receptor (EGFR) or HER family (Family 30)[154] which involved in various solid tumour such as breast tumours, colorectal cancers and gastric carcinoma.[156,157]

Although a ligand for HER-2/neu protein has not been recognised, recent published studies suggest that HER-2/neu is the preferred heterodimeric partner in the family of epidermal growth factor receptors. The tyrosine kinase activity of HER-2/neu intracellular domain triggers signal transduction pathways, which are involved in cell proliferation, migration, apoptosis, and differentiation[179].

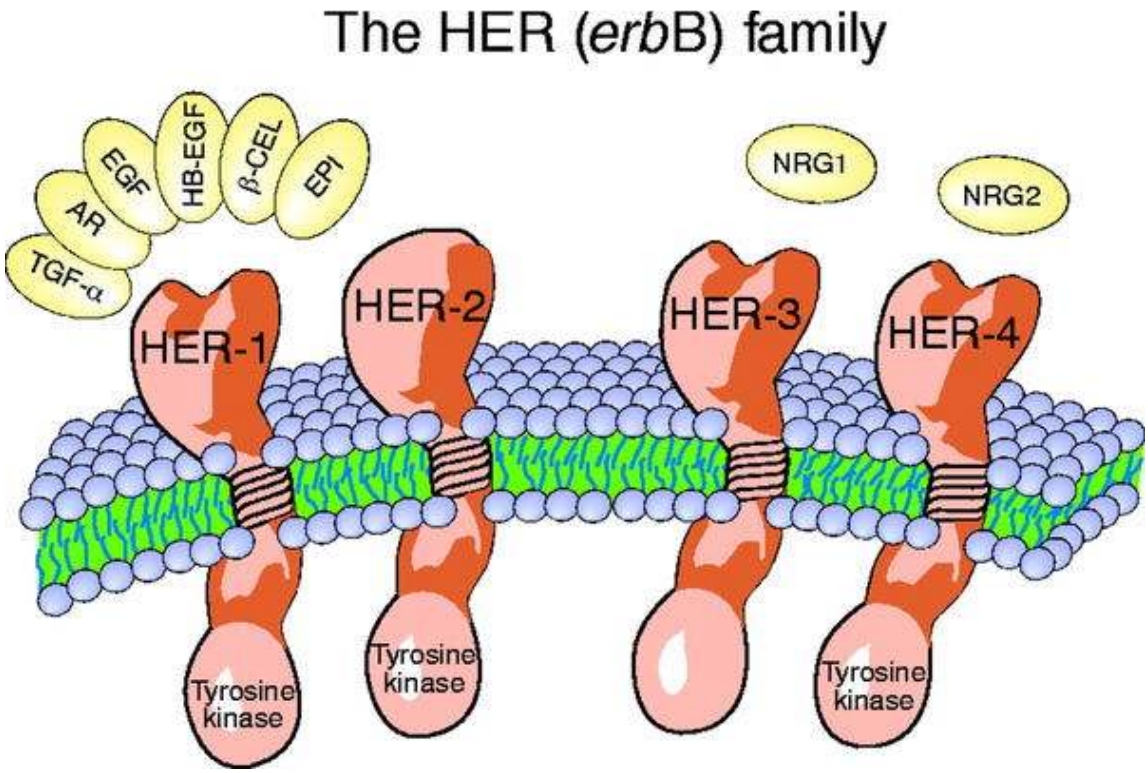


Figure 30 HER (erb) family, *HER-2/neu has no known ligands*

IMMUNOHISTOCHEMISTRY:

Albert Coons et al in 1941 first labelled antibodies directly with fluorescent isocyanate. Nakane and Pierce et al in 1966, introduced indirect labelling technique in which unlabelled antibody is followed by second antibody or substrate. Various stages of development of

Immunohistochemistry include peroxidase – anti peroxidase method (1970), alkaline phosphatase labelling (1971), avidin biotin method (1977) and two layer dextrin polymer technique (1993)[158]

ANTIGEN RETRIEVAL:

Antigen retrieval can be done by the following different techniques to unmask the antigenic determinants of fixed tissue sections.

1. Proteolytic enzyme digestion
2. Microwave antigen retrieval
3. Pressure cooker antigen retrieval
4. Microwave and trypsin antigen retrieval

PROTEOLYTIC ENZYME DIGESTION:

Huank et al in 1976 has introduced this technique to breakdown formalin cross linkages and to unmask the antigen determinants. The most commonly used enzymes include trypsin and proteinase. The disadvantage includes over digestion, under digestion and antigen destruction.

MICROWAVE ANTIGEN RETRIEVAL:

This is a new technique most commonly used in current practice. Microwave oven heating involves boiling formalin fixed paraffin sections in various buffers for rapid and uniform heating.

PRESSURE COOKER ANTIGEN RETRIEVAL:

Miller et al in 1995 compared and proved that pressure cooking method has fewer inconsistencies, less time consuming and can be used to retrieve large number of slides than in microwave method

PITFALLS OF HEAT PRETREATMENT:

Drying of sections at any stage after heat pretreatment destroys antigenicity. Nuclear details are damaged in poorly fixed tissues. Fibers and fatty tissues tend to detach from slides while heating. Not all antigens are retrieved by heat pre treatment and also some antigens like PGP 9.5 show altered staining pattern.

DETECTION SYSTEMS:

After addition of specific antibodies to the antigens, next step is to visualize the antigen antibody reaction complex. The methods employed are direct and indirect methods. In the direct method, primary antibody is directly conjugated with the label. Most commonly used labels are flouro-chrome, horse radish peroxidase and alkaline phosphatase. Indirect method is a two-step method in which labelled secondary antibody reacts with primary antibody bound to specific antigen. The use of peroxidase enzyme complex or avidin biotin complex further increases the sensitivity of immunohistochemical stains[158].

In 1993, Pluzek et al introduced enhanced polymer one step staining, in which large numbers of primary antibody and peroxidase enzymes are attached to dextran polymer back bone. This is the rapid and sensitive method[159].Dextran polymer conjugate two step visualization system is based on dextran technology in Epos system. This method has greater sensitivity and is less time consuming.

MATERIALS & METHODS

This study was proposed and conducted in the Department of Pathology of Tirunelveli Medical College Hospital after due approval of the TVMC Research Ethical Committee. The study sample comprised of gastrectomy specimens received at the Surgical Pathology division of the Department of Pathology, Tirunelveli Medical College Hospital, during the period from January 2012 to August 2014.A sample of 50 patients who were diagnosed as having gastric adenocarcinomas were selected for this study based on a set of inclusion and exclusion criteria.

INCLUSION CRITERIA

Gastrectomy specimen diagnosed as previously Gastric adenocarcinoma by endoscopic biopsy.

EXCLUSION CRITERIA

Specimens diagnosed as non epithelial tumours, secondary tumours, small cell carcinoma, squamous cell carcinoma were excluded.

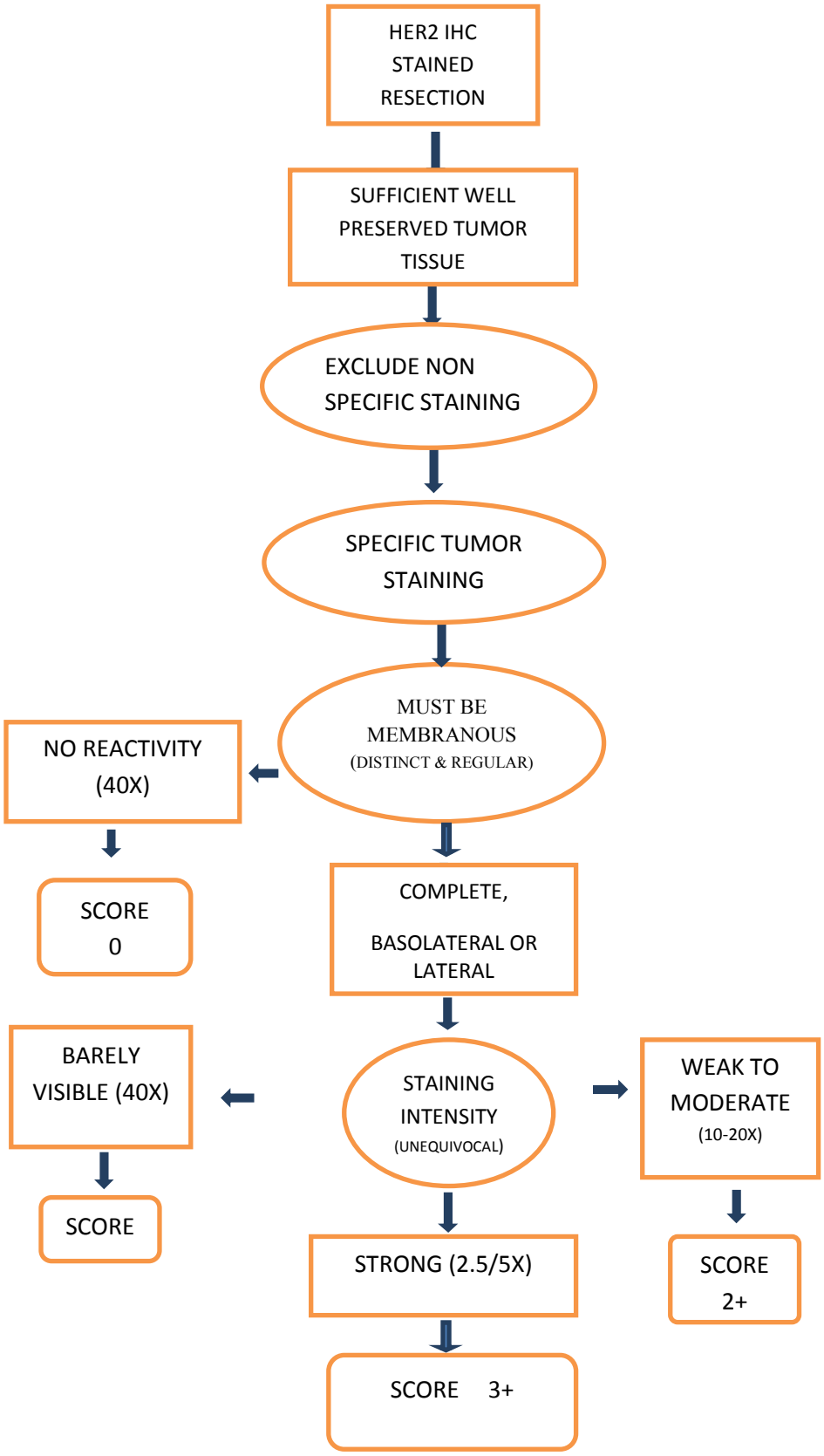
Methodology

1. Patients who had a gastrectomy done were included in this study as per inclusion and exclusion criteria and their clinical data was recorded in a piloted Pro Forma (ANNEXURE I)
2. All gastrectomy samples received were fixed in10% neutral buffered formalin as per standard procedure and processed as per standard protocols (Annexure IV), then embeddedin paraffin wax. Then 3-5 micron thickness sections were cut and stained with H&E as per standard protocols (ANNEXUREII).
3. The histological features were recorded in the study pro-forma and a detailed histomorphological diagnosis was arrived at.
4. The histomorphological data was correlated with the patient's clinical data like site of tumour, radiological details etc.
5. Tumours were histologically classified as per WHO classification.
6. The blocks were studied and representative sections were identified for immunohistochemistry.
7. Thin 3micron sections were cut and were stained with HER-2neu antibody as per standard protocol as follows (ANNEXURE IV).

Consensus recommendations on HER-2 scoring for gastric cancer.	**(Table6)**
Definition	Score
No reactivity or membranous reaction in <10% of cells	0 / negative
Faint /barely perceptible membranous reactivity in >10% of cells Cells are reactive only in part of their membrane	1+ /negative
Weak to moderate complete or basolateral membranous reactivity in >10% of cells	2+ /equivocal
Moderate to strong complete or basolateral membranous reactivity in >10% of cells	3+ /positive

A strong brown staining was located in cell membrane of malignant cells using this staining method. The Thermo Fisher Scientific (Cheshire, UK) Test Protocol System was used to grade the membrane staining. We used membrane stain graduation scale for assess the IHC. It is graded 0, 1+, 2+, 3+ according to the panel scoring (Table 6)[160]. And approach to the standardized IHC scoring in gastric cancer summarised.(Figure 31)[161] The IHC stained sections was examined and evaluated manually by 2 pathologists.

FIG 31 APPROACH TO IMMUNOHISTOCHEMISTRY SCORING IN GASTRIC CANCER SAMPLE



Statistical analysis

The correlations between HER2/neu status and patient clinico pathological data was evaluated by using fisher t test and chi- square test. p value less than 0.05 considered to be positive. Data has been analyzed by using the SPSS statistical software program (Version 19.0)for Microsoft Windows 7.

OBSERVATION AND RESULTS

Gastrectomy samples of 57 patients for gastric carcinomas were received at the surgical pathology division of the Department of Pathology between January 2012 and August 2014. Of these 57 patients , 52 (91.2%) had gastric adenocarcinoma in histopathological examination.(Table 7)

TABLE 7

Gastrectomy Specimens Received(n)	**57**
Gastric Adenocarcinoma(n)	**52**
GIST(n)	**3**
Gastric Non Hodgkins Lymphoma(n)	**2**

Of the 52 patients with adenocarcinoma of the stomach, 2 patients were not included in further study. Two cases excluded from the study because of non availability of pathological blocks. (Table8)

TABLE 8: THE STUDY GROUP

Primary Gastric Adenocarcinoma(n)	**52**
No Of Cases Selected For Study (*n*)	**50**
No Of Cases Not Selected For Study (*n*)	**2**

These 50 cases were studied for Her 2/neu expression. Her 2/neu expression was found to be correlated with the clinicopathologic parameters. Of these 50 cases,45 cases comprised of subtotal gastrectomies and the remaining cases were subtotal gastrectomies as mentioned on Table 9.

TABLE 9: TYPE OF SURGERY

Gastrectomy	**Total cases(*n*)**
Subtotal	**44**
Total	**6**
TOTAL	**50**

Of these 50 cases which were studied for the HER2/neu reactivity, 4 cases showed 3+ (8%) , 4 cases showed 2+ (8%) , 16 cases showed 1+(32%), and 26 cases (52%) did not show reactivity (negative) as per the scoring system devised by Hofmann scoring system[177](Table 10)

TABLE 10. HER2/neu REACTIVITY IN THIS STUDY

HER2/*neu* score	**Number of cases (*n)***	**Percentage(%)**
0	**26**	**52**
1+	**16**	**32**
2+	**4**	**8**
3+	**4**	**8**

TABLE 11: GENDER OF PATIENTS &HER2 neu EXPRESSION

Gender	Total cases(*n*)	HER2 neu negative(*n*)	HER2 neu positive(*n*)
Male	**34**	**29**	**5**
Female	**16**	**13**	**3**
Total	**50**	**42**	**8**

Of the 50 cases of gastric adenocarcinoma, 34 cases were male and 16 were female. Out of 34 male cases, 5 cases (14.7%) were HER2/neu positive. Of the 16 female cases, 3 cases (18.75%) were positive for HER2/neu expression.(Table 11& Chart 1)

TABLE 12 : AGE OF PATIENTS &HER2/neu EXPRESSION

Age	Total cases(*n)*	HER2 neu negative(*n*)	HER2 neu positive(*n*)
<57	**22**	**20**	**2**
≥57	**28**	**22**	**6**
Total	**50**	**42**	**8**

Among the 50 cases, 22 patients were less than median age of 57 years and of which 2 cases (9.1%) positive for HER2/neu. 28

patients were above the median age, of which 6 cases were positive (21.43%) for HER2/neu expression.(Table 12& Chart 2)

TABLE 13: LOCATION OF TUMOUR &HER2/neu EXPRESSION

Tumour Location	Total cases(*n*)	HER2 neu negative(*n*)	HER2 neu positive(*n*)
Body & OGJ	**17**	**11**	**6**
Pyloric antrum	**33**	**31**	**2**
Total	**50**	**42**	**8**

Among the 50 cases, in 17 cases tumour were located in corpus and OGJ, of which two cases tumours originated from oesophagogastric junction. From these 6 cases (35.3%) found positive for HER 2/neu expression. In 33 cases tumour were located in pyloric antral region, of which 2 cases (6.06%) were positive for HER2/neu expression.(Table 13& Chart 3)

TABLE 14: TUMOUR SIZE&HER2/neu EXPRESSION

Tumour Size	Total cases(*n*)	HER2 neu negative(*n*)	HER2 neu positive (*n*)
<5 cm	**22**	**19**	**3**
≥ 5 cm	**28**	**23**	**5**
Total	**50**	**42**	**8**

The gastrectomy specimen examined, and it was correlated that of the 50 patients studied 22 patients had a primary tumour size of less than that of median tumour size 5cm, of which 3 cases(13.6%) shows positive for HER2/neu expression. 28 patients had a primary tumour size of more than 5cms, of which 5 cases(17.9%) shows positive for HER2/neu expression.(Table 14& Chart 4)

TABLE 15: LAUREN'S CLASSIFICATION & HER2/neu EXPRESSION

Lauren's classification	Total cases(n)	HER2 neu negative(n)	HER2 neu positive(n)
Intestinal	**32**	**28**	**4**
Diffuse	**18**	**14**	**4**
Total	**50**	**42**	**8**

50 Cases of gastric adenocarcinoma were grouped into 2 according to Lauren's classification, out of which 32 cases belonged to intestinal type and 18 cases belonged to diffuse type. Out of the 32 cases of intestinal type , 4 cases (12.5%) found to be positive for HER2/neu expression. And 4 (22.22%) cases out of 18 cases of diffuse type shows positivity for HER2/neu expression.(Table 15& Chart 5)

TABLE 16 : WHO CLASSIFICATION & HER2neu EXPRESSION

HISTOLOGIAL TYPE	**TOTAL CASES(*n*)**	**HER2 neu NEGATIVE(*n*)**	**HER2 neu POSITIVE(*n*)**
TUBULAR	23	20	3
PAPILLARY	7	6	1
MUCINOUS	2	2	0
SIGNET	10	6	4
DIFFUSE	8	8	0

Among histological forms, 13% of tubular carcinomas, 14.2% of papillary carcinomas, 40% of signet ring cell carcinomas showed HER2neu expression. Mucinous carcinoma and diffuse type carcinoma showed negative for HER2neu expression. (Table 16 and Chart 6)

Table 17: LYMPH NODAL STATUS &HER2/neu EXPRESSION

Nodal status	**Total cases(*n*)**	**HER2 neu negative(*n*)**	**HER2 neu positive(*n*)**
N0	13	13	0
N1	14	12	2
N2	16	11	5
N3	7	6	1

The gastrectomy specimen were examined and correlated. Among these 50 patients studied, 13 patients had no nodal involvement (N0) and none of the cases were shown HER2/neu positivity. 14 cases had N1 level lymph node metastasis, of which 2 cases(14.3%) shows positive for HER2/neu expression. 16 cases had N2 level lymph node metastasis, of which 5 cases(31.25%) positive for HER2/neu expression. 7 cases had N3 level lymph node metastasis, of which 1 cases(14.28%) positive for HER2/neu expression.(Table 17& Chart 7)

TABLE 18: LYMPH NODE RATIO &HER2/neu EXPRESSION

Lymph node ratio	**Total cases(*n*)**	**HER2 neu negative(*n*)**	**HER2 neu positive(*n*)**
<0.5	**23**	**21**	**2**
≥0.5	**27**	**21**	**6**
Total	**50**	**42**	**8**

Lymph Node Ratio (LNR) defined as the ratio of lymph nodes with tumour metastasis to the total lymph nodes dissected.

The median of the total received lymph nodes were found to be 5, and the median number of involved lymph nodes were 2. The ratio of the involved and uninvolved lymph node was 0.5. Among 50 cases, 23 cases had 0.5 LN ratio, of which 2 cases (8.7%) showed positivity for HER2/neu expression. And 27 cases had more than 0.5 LN ratio, of which 6 cases (22.22%) showed positivity for HER2/neu protein. (Table 18& Chart 8)

TABLE 19: RESECTED MARGINS&HER2 neu EXPRESSION

Resection Margin	**Total cases *(n)***	**HER2 neu negative(*n*)**	**HER2 neu positive(*n*)**
R0	**30**	**26**	**4**
R1	**1**	**1**	**0**
R2	**19**	**15**	**4**
TOTAL	**50**	**42**	**8**

Out of the 50 cases studied, 30 cases shows no tumour involvement in the resected margins. Among these 30 cases, HER2/neu expression was observed in 4 cases (13.33%). Only one case had shown a feature of microscopic positivity for tumour(R1), of which

none of the cases showed HER2/neu expression.19 cases had shown gross residual disease (R2), of which 4 cases(21.05%) were positive for HER2/neu expression.(Table 19& Chart 9)

TABLE 20: LYMPHATIC INVASION&HER2 neu EXPRESSION

Lymphatic invasion	Total cases*(n)*	HER2 neu negative (*n*)	HER2 neu positive(*n*)
NO	**16**	**15**	**1**
YES	**34**	**27**	**7**
TOTAL	**50**	**42**	**8**

Lymphatic invasion characterized as a embolus of carcinoma cells or infiltration of carcinoma cells as single/ tiny clusters in lymphatic capillaries or interstitial space of perilymphnodal fat tissue Among 50 cases of gastric adenocarcinoma, 34 cases showed lymphatic invasion. Out of 34 cases, HER2/neu expression was noted in 7 cases (20.6%). And 16 cases without lymphatic invasion also shows 6.25% (1 case) positivity for HER2/neu expression.(Table 20& Chart 10)

TABLE 21: SEROSAL INVASION &HER2 neu EXPRESSION

Serosal Invasion	Total cases(*n*)	HER2 neu negative(*n*)	HER2 neu positive(*n*)
No	**27**	**21**	**6**
Yes	**23**	**21**	**2**
Total	**50**	**42**	**8**

Serosal invasion characterized as tumour invading into visceral peritoneum or tumour present at the peritoneal surface with inflammatory

reaction/mesothelial hyperplasia/ ulceration or tumour cells demonstrated free in peritoneum with the evidence of adjacent ulceration.

Among 50 cases with gastric adenocarcinoma, 2 out of 23 cases (8.7%) with serosal invasion were positive for HER2 expression, while 6 out of 27 cases (22.22%) without serosal invasion showed positivity for HER2 expression. (Table 21& Chart 11)

TABLE 22 : PERINEURAL INVASION &HER2 neu EXPRESSION

Perineural Invasion	**Total cases(*n*)**	**HER2 neu negative (*n*)**	**HER2 neu positive (*n*)**
No	**36**	**32**	**4**
Yes	**14**	**10**	**4**
Total	**50**	**42**	**8**

Perineural invasion (PNI) is a process of neurotropic carcinomatous spread and perineural spread.

Among 50 cases with gastric adenocarcinoma, 4 out of 14 cases (28.6%) with perineural invasion were positive for HER2 expression, while 4 out of 36 cases(11.11%) without perineural invasion showed positivity for HER2 expression.(Table 22& Chart 12)

TABLE 23: SIGNET CELL CARCINOMA &HER2 neu EXPRESSION

Histological type	Total cases(*n*)	HER2 neu negative(*n*)	HER2 neu positive(*n*)
SRC	40	36	4
NON SRC	10	6	4
Total	50	42	8

Signet ring carcinoma (SRC) defined as an adenocarcinoma in which predominant component (more than 50% of the tumour cells)made up of isolated or small groups of neoplastic cells containing intracytoplasmic mucin.

Among 50 cases of gastric adenocarcinoma, 4 out of 40 cases (10%) without signet cell features were defined as positive for HER2 expression, while 4 cases out of 10 cases (40%) with signet cell features showed positivity for HER2 expression .(Table 23& Chart 13)

TABLE 24 : TNM STAGING &HER2 neu EXPRESSION

TNM stage	Total cases(n)	HER2 neu negative (n)	HER2 neu positive (n)
stage I	6	6	0
stage II	24	21	3
stage III	20	15	5
Total	50	42	8

TNM (Tumour- Node-Metastasis) Staging is the widely used, recommended staging system for gastric cancers. It has been revised

according to UICC guidelines(7th edition). This new classification was based on the number of positive regional lymph nodes instead of the anatomic location of the regional lymph node metastasis, which was mentioned in (Annexure III)

Of the 50 cases studied all 50 patients (100%) had gastric adenocarcinoma. According to seventh edition of UICC guidelines, pTNM staging of 50 cases were done[189]. Stage evaluation revealed that 6 cases falls under stage I, 24 cases in stage II & 20 cases in stage III. 5 out of 20 cases in stage III(25%) had shown positive for HER 2 expression. 3 out of 24 cases(12.5%) in stage II were defined positivity for HER2 expression, while none of the stage I cases revealed HER2 expression.(Table 24& Chart 14)

CLINICPATHOLOGICAL PATIENT CHARACTERISTICS (Table 25,26,27,28)

In this study all 50 cases fulfilled study criteria were included. Clinicopathological characteristics are mentioned in following tables.

PATIENT CHARACTERISTICS (TABLE 25)	***n* =50**
Patients (*n*)	50
Age(years)	
Mean	55.66±10.53
Median	57
Gender, *n (%)*	
Men	34 (68)
Women	16 (32)
Lauren phenotype, *n (%)*	
Intestinal	32 (64)
Diffuse	18 (36)
Histological type, *n (%)*	
Tubular	23(46)
Papillary	7(14)
Mucinous	2(4)
Signet	10(20)
Diffuse	8(16)

PATIENT CHARACTERISTICS (TABLE 26)	***n* =50**
Localisation, *n (%)*	
Body& OGJ	17 (30)
Pyloric antrum	33(64)
Resected Margins, *n (%)*	
pR0	30 (60)
pR1	1 (2)
pR2	19 (38)

PATIENT CHARACTERISTICS (TABLE 27)	***n* =50**
TNM stage(7^{TH} edition) , *n (%)*	
Stage I	6 (12)
Stage II	24 (48)
Stage III	20 (40)

PATIENT CHARACTERISTICS (TABLE 28)	***n* =50**
Resected lymph nodes	
Mean ± SD	5.6±3.94
Median,n	5
Positive lymph nodes	
Mean ± SD	3.1±3.55
Median,n	2
Lymph node ratio	
Median,n	0.5
Lymphatic invasion, *n (%)*	
pL0	16 (32)
pL1	34 (68)

The median number of resected lymph nodes were 5, and the median number of involved lymph nodes were 2. The ratio between metastatic and resected lymph nodes (N ratio) has been tabulated. Median of lymph node ratio was 0.5

CLINICOPATHOLOGICAL VARIABLES OF GASTRIC CANCER PATIENTS STRATIFIED BY HER-2/NEU STATUS (Table 29)

The medical records of each case was reviewed and medical , demographic and pathological data of the patients was collected. Patient age and sex were included in demographic data. Tumour location and TNM stage and treatment history were included in Clinical and pathological data. **Table 29**

Parameter	**HER 2neu Negative (n)**	**HER 2neu Positive (n)**	**P-Value**
Age (years)			**0.439**
>57	20	2	
≥57	22	6	
Gender			**0.699**
Men	29	5	
Women	13	3	
Localisation			**0.027**
Body & OGJ	11	6	
Pyloric antrum	31	2	
Tumor Size			**1.000**
<5cm	19	3	
≥5cm	23	5	
Differentiation			**1.000**
Yes	22	4	
No	20	4	
Lauren's Classification			**0.4357**
Intestinal	28	4	
Diffuse	14	4	
WHO Type			**0.174**

Tubular	20	3	
Papillary	6	1	
Mucinous	2	0	
Signet	6	4	
Diffuse	8	0	
Nodal Status			**0.157**
No	13	0	
N1	12	2	
N2	11	5	
N3	6	1	
Lymph node ratio			**0.2609**
<0.5	21	2	
≥0.5	21	6	
Perineural Invasion			**0.1968**
No	32	4	
Yes	10	4	
Lymphatic Invasion			**0.4092**
No	15	1	
Yes	27	7	
Serosal Invasion			**0.2609**
No	21	6	
Yes	21	2	
Resected Margins			**0.7011**
R0	26	4	
R1	1	0	
R2	15	4	
TNM stage (7^{th} Edition)			**0.277**
Stage I	6	0	
Stage II	21	3	
Stage III	15	5	

This study consist of 31 males and 19 females with ages ranging between 37 and 80 years . 57 years stratified as a median tumour age according to this study.(Refer table no 8).

Tumour size of 5cm stratified as median tumour size according to this study.(Refer table no 9)

The ratio of metastatic to examined lymph nodes (LNR) was determined, and HER2neu expression is analysed in relation to two groups LNR < 0.5, and LNR > 0.5. (Refer table no 13).

HER 2/*neu* expression is correlated with clinicopathologic parameters. The 50 gastric cancer gastrectomy tissue specimens were examined for the presence of HER 2/*neu,* determined by immunohistochemistry. Of these specimens, 6 (12%) exhibited HER2/*neu*- positive expression.

1. HER 2/neu expression was correlated with Histological type- signet ring cell carcinoma with p value < 0.05.
2. HER 2/neu expression was also correlated with location of the tumour. OGJ and corpus tumours are associated with HER2 neu expression compared with antral- pyloric tumour (p value < 0.05)
3. There were no differences between the groups in terms of age, gender, type of gastrectomy, tumour size, nodal status, stage of disease, perineural invasion, serosal invasion and differentiation of tumour.

CHART 1-GENDER Vs HER2 EXPRESSION

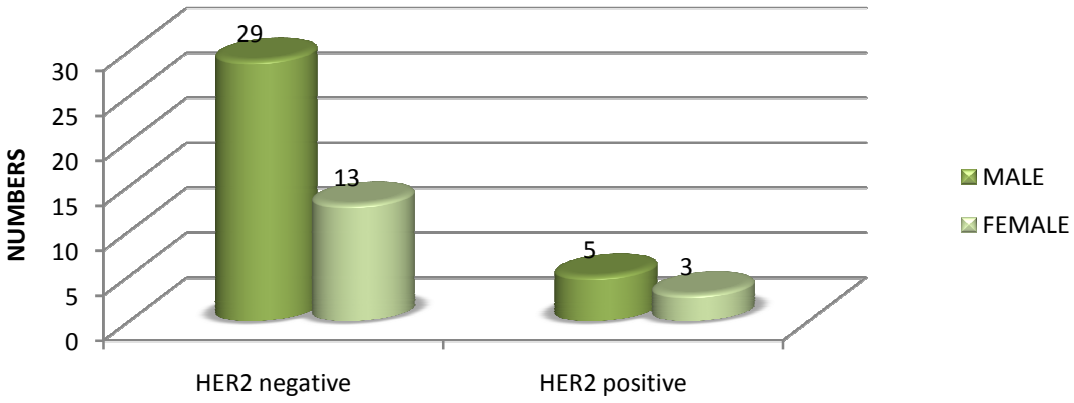


CHART 2 -AGE Vs HER2 EXPRESSION

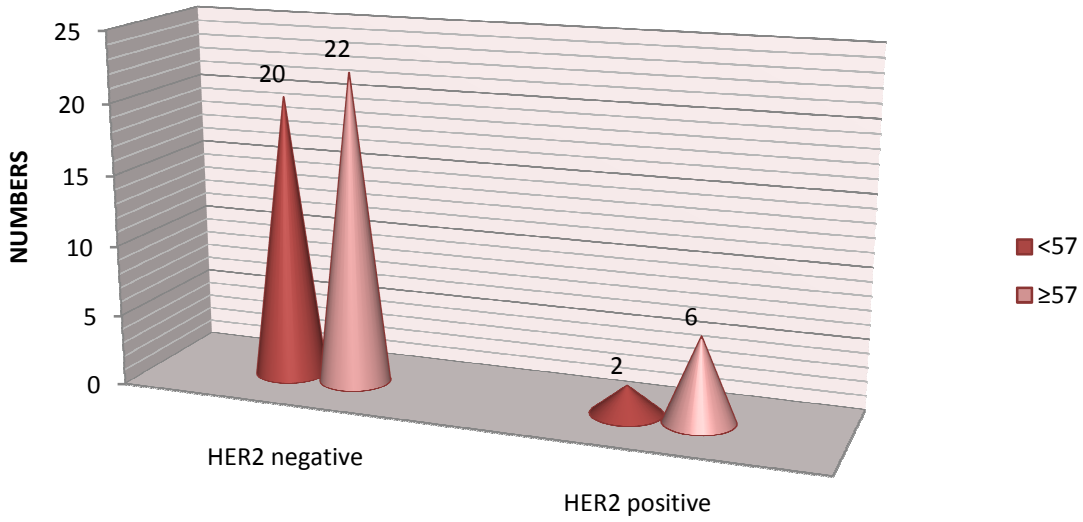


CHART 3 - TUMOUR LOCATION Vs HER2 EXPRESSION

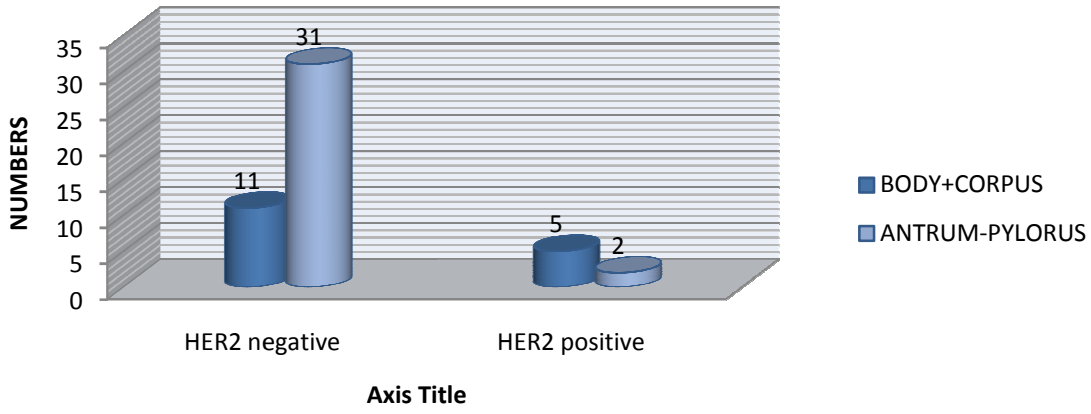


CHART 4 - TUMOUR SIZE Vs HER2 EXPRESSION

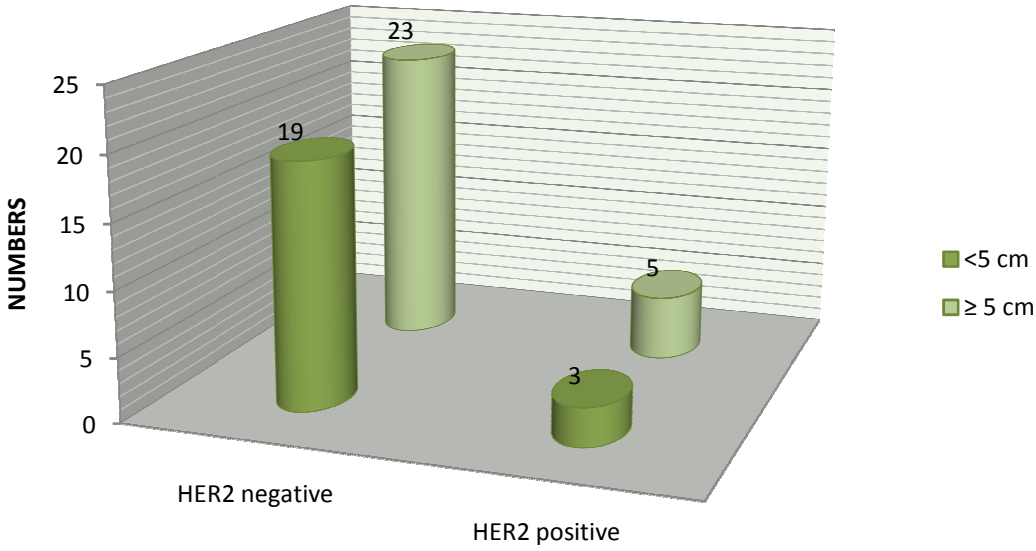


CHART 5- LAUREN'S TYPE Vs HER2 EXPRESSION

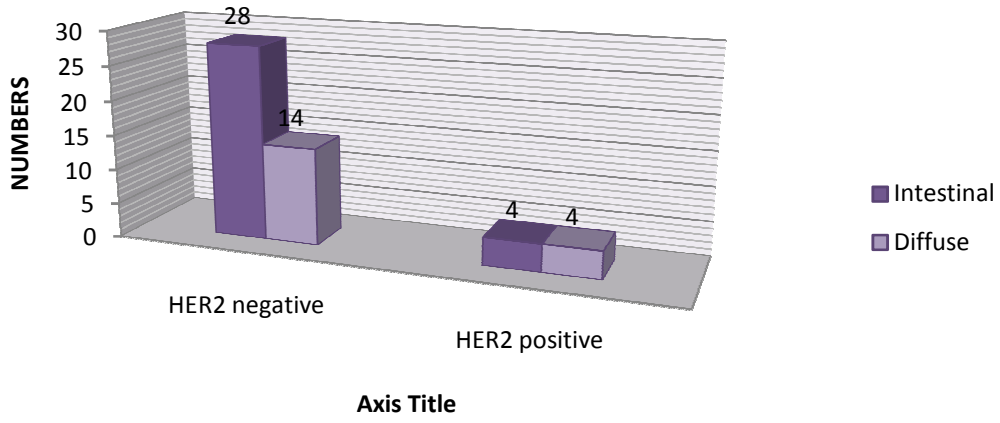


CHART6- WHO Vs HER2neu EXPRESSION

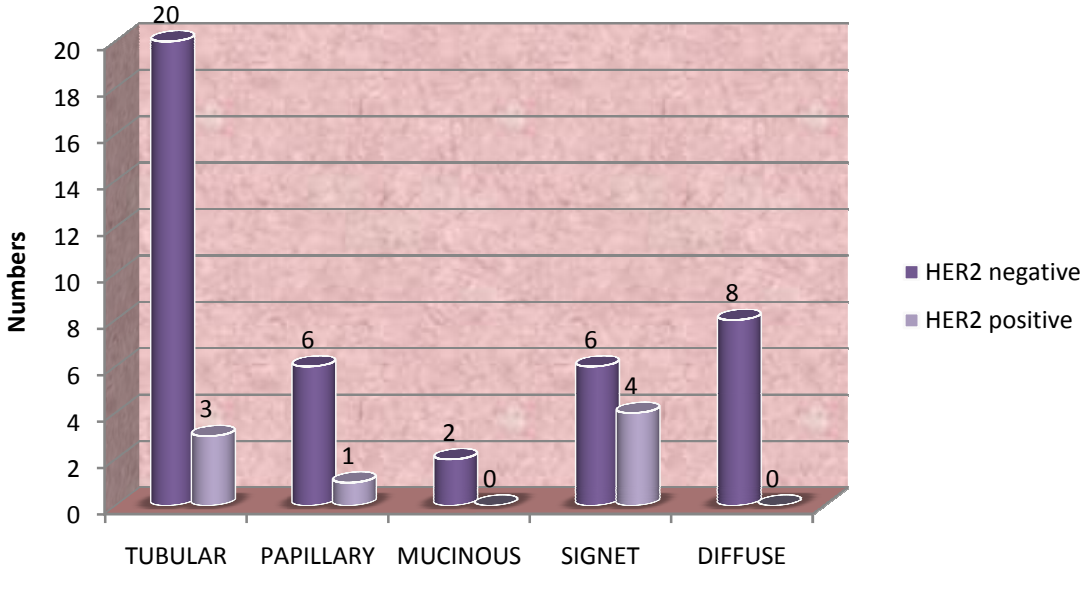


CHART 7-NODAL STATUS Vs HER2 EXPRESSION

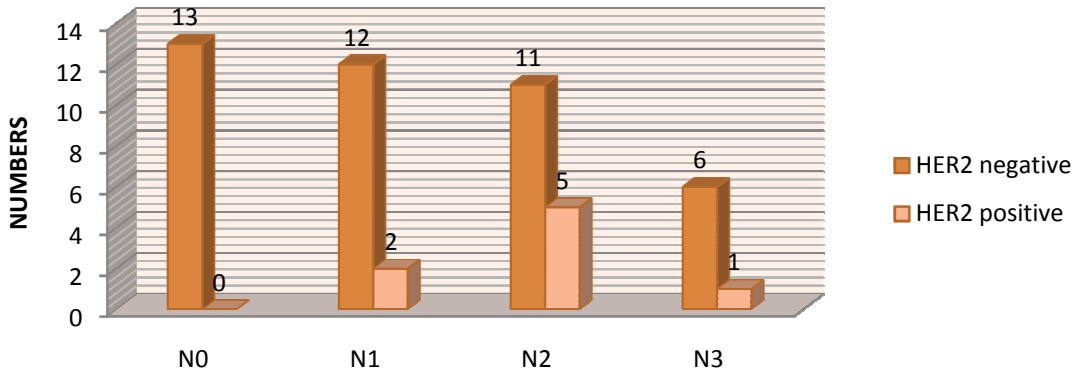


CHART 8- LYMPH NODE RATIO Vs HER2 EXPRESSION

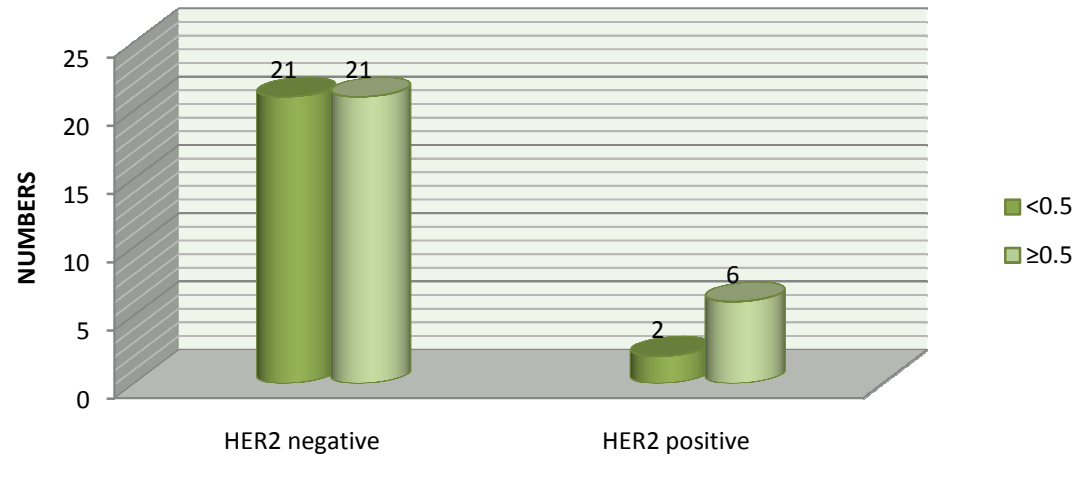


CHART 9- MARGINAL STATUS Vs HER2 EXPRESSION

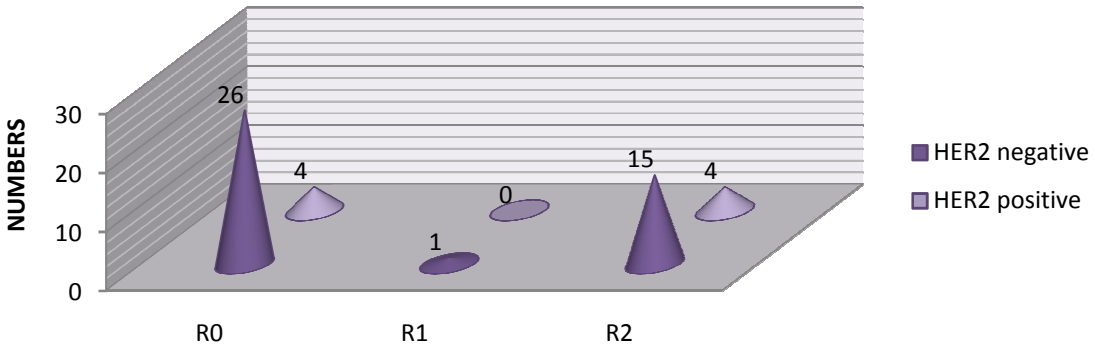
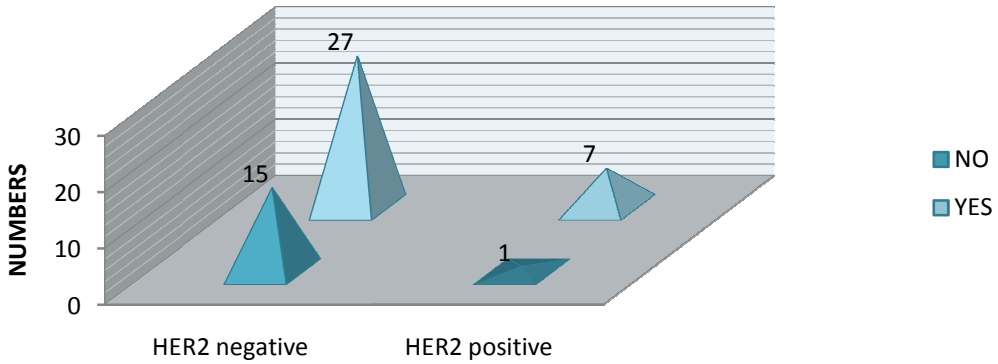


CHART 10- LYMPHATIC INVASION Vs HER2 EXPRESSION



\

CHART 11 - SEROSAL INVASION Vs HER2 EXPRESSION

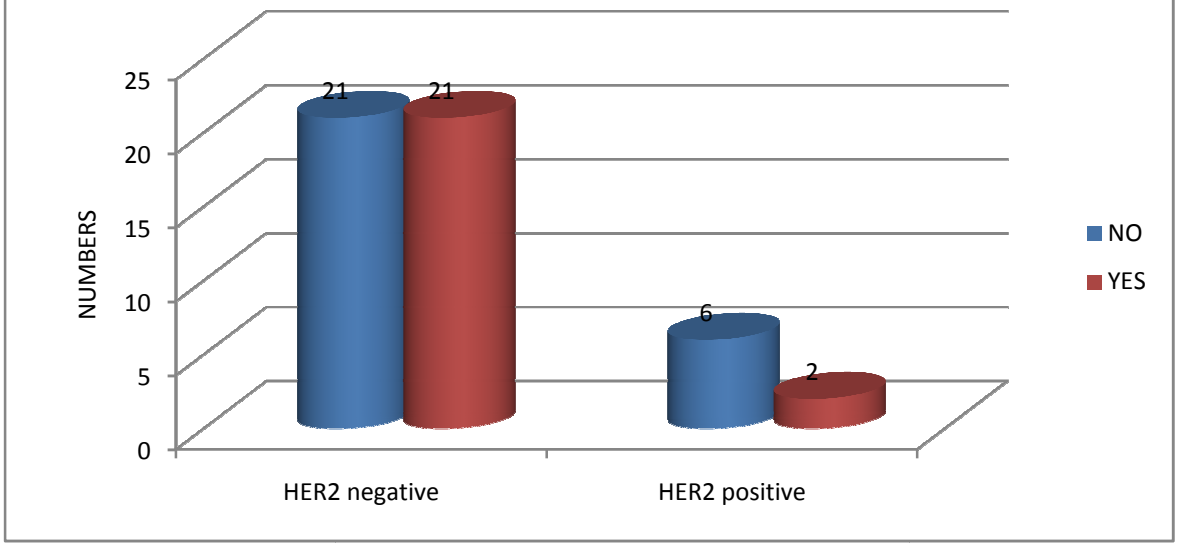


CHART 12- PERINEURAL INVASION Vs HER2 EXPRESSION

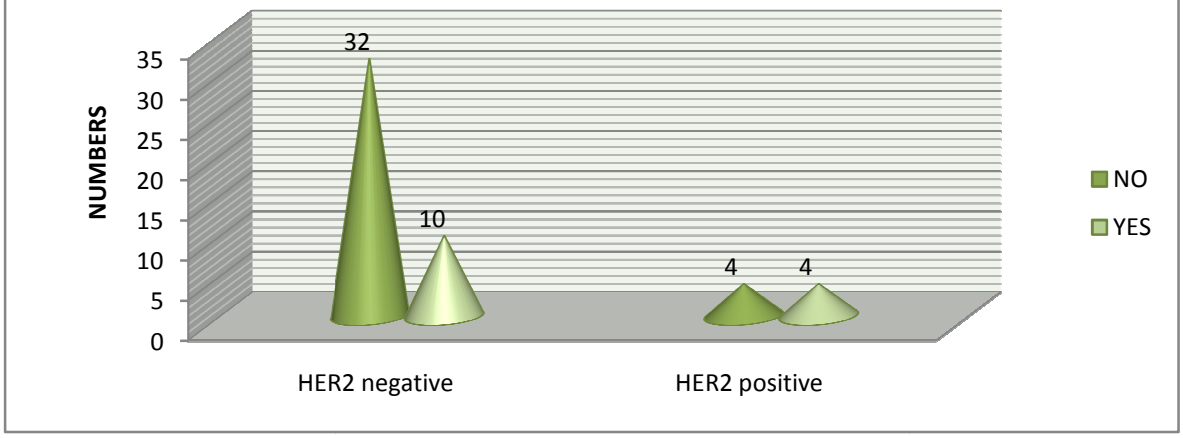


CHART 13 - SIGNET RING CARCINOMA Vs HER2 EXPRESSION

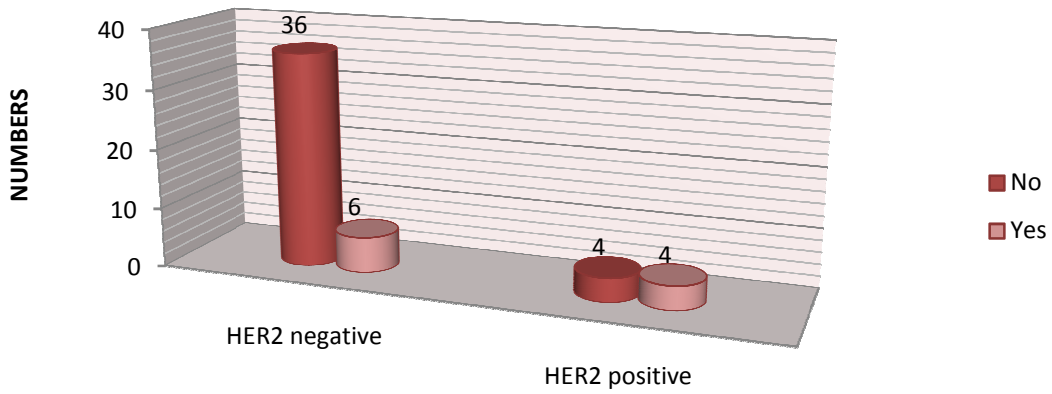
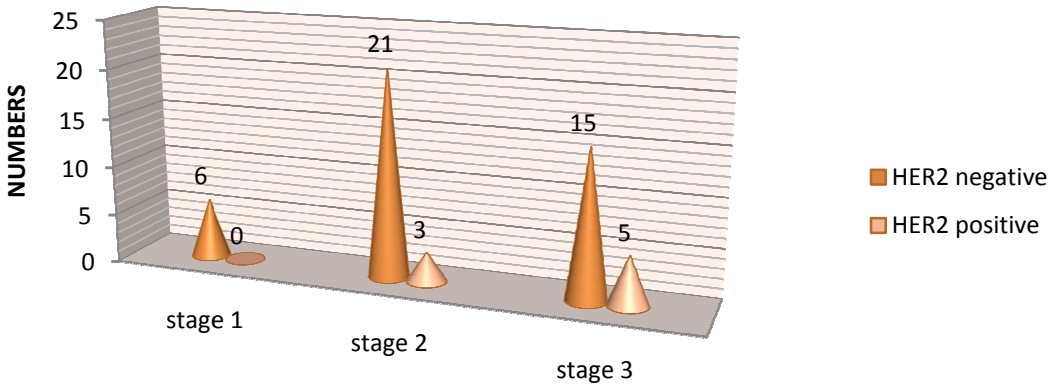


CHART 14 - TNM STAGE Vs HER2 EXPRESSION



DISCUSSION

HER2/neu and its ligands are frequently overexpressed in human cancers. HER2/*neu* protein is member of the epidermal growth factor receptor (EGFR) family,[162] and it is a 185 kDa transmembrane tyrosine kinase (TK) receptor . HER2/neu dimerizes with either itself or a structurally similar HER2/neu family member to activate a cascade of cellular pathways that contribute to cell growth, proliferation & survival. In breast carcinoma , HER 2/neu amplification / over expression has been associated poor overall prognosis , and it shows resistance to standard cytotoxic therapies and susceptibility to HER2/neu - targeted therapy such as transtuzumab. Thus in breast carcinoma, HER2/neu is a biomarker that has both prognostic and predictive value[163]. Although various therapies for gastric carcinoma are available, such as gastrectomy with extensive lymphadenopathy and surgery combined with chemotherapy, the patients respond poorly to the conventional treatment. In recent years, molecular target therapy is a new treatment modality for gastric cancer and HER2/neu has been identified as a potential therapeutic target. Over expression of HER 2/neu receptor in gastric carcinoma using immunohistochemistry is detected in 1986 and has been recognised as an important prognostic factor[164].

The survival time of patients with breast carcinoma and positive HER2/neu disease is significantly shorter than that those with HER2/neu negative tumour.[165,166]Thus detection of HER2/neu status is of greater significance in diagnosis of gastric carcinoma.

The pressing clinical question is whether or not HER2/neu expression confers prognostic information. Over expression of HER2/neu in gastric cancer varies from 8.2% to 62.5% in different reports.[167] Some studies revealed HER2/neu appears to be a valuable prognostic factor.[16,168] However , the clinical significance of this finding yet to be clearly defined. In literature, few studies have shown a strong association between HER2/neu over expression and worse prognosis.[169]

Due to genetic heterogeneity of gastric carcinoma, the HER2/neu over expression and amplification are different when compared to breast carcinoma. This variation may be explained by different sample size, and also other possible reasons for this wide variation includes pre analytical variables, such as differences in fixation technique, antibodies , scoring system, staining methods, subjectivity of pathologist interpretation and intra tumoural staining heterogeneity. Studies are needed for evaluating the validity of commercially available antibodies.

In this study, over expression of HER2/*neu* protein observed in 8 of 50 gastric carcinoma specimens as determined by IHC. According to this findings, 16% of patients expressed HER2/*neu* protein by IHC.

In this study, we have found a statistically significant association between positivity for HER2/neu and tumour location. It shows correlation with higher rate of HER2/neu positivity in OGJ & corpus cancer than antral-pylorus cancer (35.29% Vs 6.06% respectively) with a p value <0.05, and it is consistent with results of other studies.

M.Tanner et al (2005)[170] found that positive rates of HER2/neu ranges from 24% to 12% for tumours located at the gastro oesophageal junction or in other areas of the stomach, respectively. The 2009 TOGA (Trastuzumab for Gastric Cancer) trial also revealed in the same way and showed a positivity which ranges between 32% and18%, respectively.[171]

In contrast, Marx Andreas H., et al (2009) found that there was no statistically significant difference between HER2/neu positivity and tumour site.[172]

In these we have also found statistically significant association between signet ring cell carcinoma and HER2/neu expression (40%), with p value of <0.05. Conflicting evidence regarding HER2 and signet ring cell features exists.

Cangiano J et al (2008)[173] study found that tumours showing signet ring cell features uniformly overexpressed HER2/neu as measured by IHC, although the sample size was not reported and determination of HER2 protein status relied on the breast cancer scoring system for IHC without molecular analysis (FISH). Future studies should make an effort to quantify the association between signet ring carcinoma and HER2/neu status.

In contrast, Grabsch H et al (2010)[167] reported that there is no significant correlation between signet cell features and HER2/neu expression.

In this study there was no significant correlation between HER2neu expression and the clinicopathologic prognostic factors , such as age, gender, similar to the studies done by M.Tanner et al (2005)[170],AnanievJulian, et al (2011).[174]

In this study, no relationship was found between HER2neu expression and primary tumour size, TNM staging, nodal status, lymph node ratio and it is consistent with of other studies. Ghaderi, Abbas, et al(2002) reported that HER2 neu overexpression was not associated with tumour stage[175]

Halon Agnieszka, et al (2012) observed that there was no relationship found between HER2neu and primary tumour size and

degree of spread to regional lymph nodes.[176] HER2neu expression was evaluated by immunohistochemical method and the results were similar to our study.

In contrast, Wang Yuan-Yu, et al (2011) Positive expression of HER2neu correlated with age, size of tumour, location of tumour, depth of invasion, vessel invasion, lymph node, and distant metastasis and TNM stage[177]

GZ Yu et al (2009) also observed significant differences in HER2neu expression between the primary tumours and the lymph node metastases ($P < 0.01$). Overexpression ofHer2 was associated with age (>60 years), tumour location (cardia of stomach), adenocarcinoma, and high/moderate differentiation[184.]

We did not observe a statistically significant correlation between HER2/neu overexpression and Lauren's classification , which has a prognostic significance. C. Gravalos and A. Jimeno (2008), reported the relationship between HER-2/neu overexpression and Lauren's classification of tumours[178]. But this correlation was not statistically significant. Y Kang et al (2008)reported that HER-2/neu overexpression correlated with the histological type as in Lauren's classification with 34% intestinal type; 6% diffuse being HER2/neu positive[179].

Due to significant differences in the historical studies, the role of HER2/neu as a prognostic marker in gastric carcinoma has been controversial. More recent studies found that HER2/neu is an important poor prognostic factor in gastric cancer patients.[180-183] This study concludes that HER2/neu status has a significant role in prognosis of gastric carcinoma and may be considered as an independent prognostic factor in gastric carcinoma patients. So further study is needed to explain the role of HER2/neu on development and prognosis of gastric cancer.

SUMMARY

1. HER2 neu expression in Gastric adenocarcinoma is 16%.
2. Her2 neu expression is more in Oesophageal gastric junctional & corpus location of tumours (35.3%) compared to Pylorus-Antral tumours.
3. Her2 neu expression is more in patients with signet ring cell carcinoma (40%) compared to Non signet ring cell carcinoma.

CONCLUSION

1. HER2neu expression in Gastric cancer has a role to play as an independent prognostic factor and requires additional studies.
2. This study concludes that the Histological type of signet ring cell carcinoma, act as a as an independent prognostic factor.
3. Tumour located in Oesophageal gastric junction & corpus of the stomach is the candidate parameter for prognostication.

GASTRIC ADENOCARCINOMA – INTESTINAL TYPE

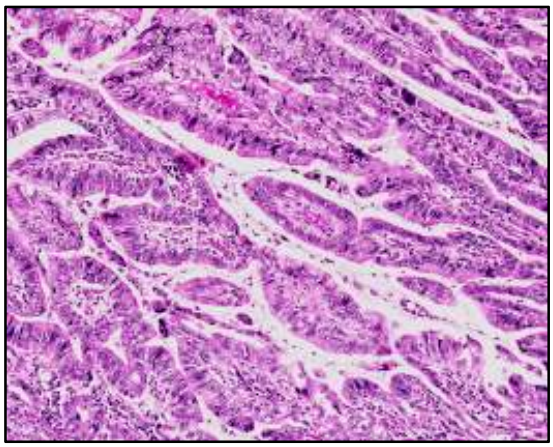


Figure 31:IT – well differentiated with well sheets formed glands (100X) HPE- 3254/13

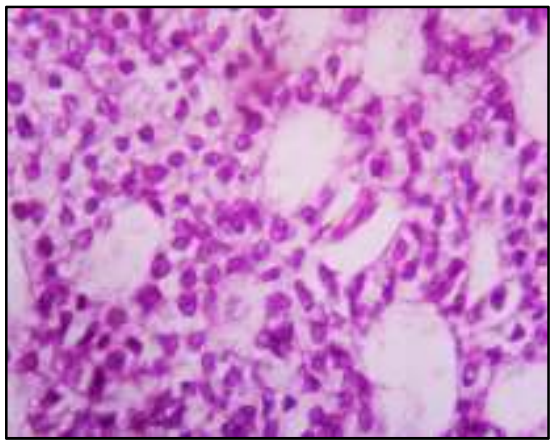


Figure 32: Malignant epithelial cells in with nuclear pleomorphism and prominent nucleoli (400X) HPE- 3254/13

GASTRIC ADENOCARCINOMA – DIFFUSE TYPE

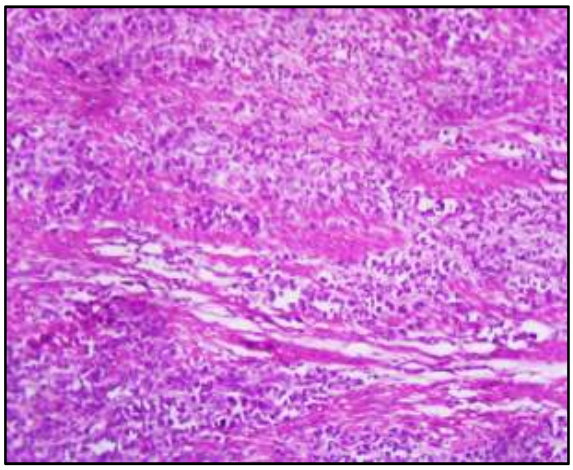


Figure 33: Poorly cohesive cells diffusely infiltrating the gastric wall. (100X) HPE-1992/14

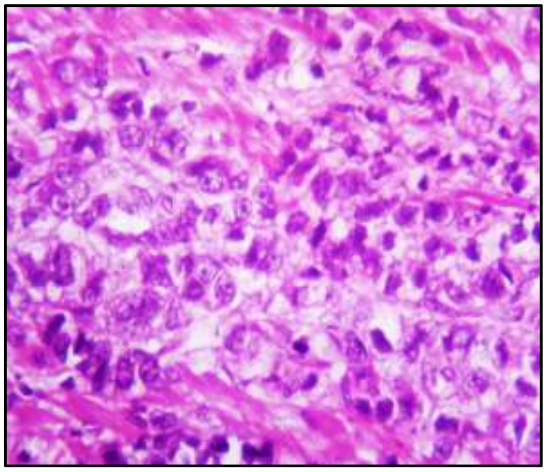


Figure 34 : Pleomorphic and round poorly cohesive cells (400X) HPE- 1992/14

TUBULAR ADENOCARCINOMA

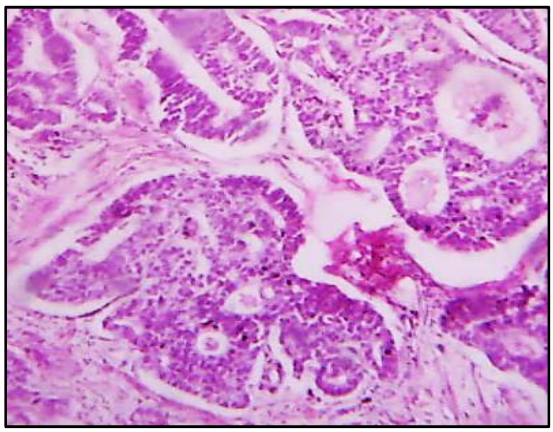


Figure 35 : Numerous tubules of varying Size (100 X) HPE- 108/ 14

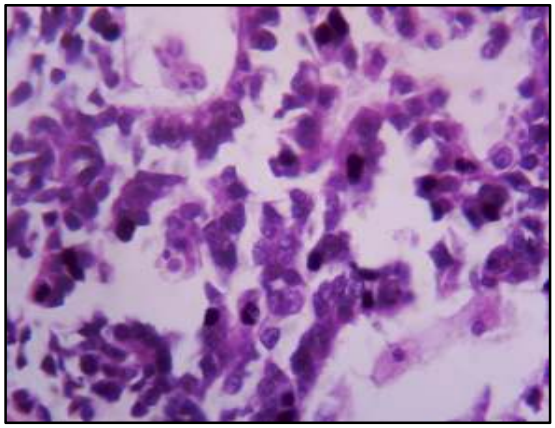


Figure 36 : Tubules lined by cuboidal to columnar cells with cytological atypia (400 X) HPE-108 /14

PAPILLARY ADENOCARCINOMA

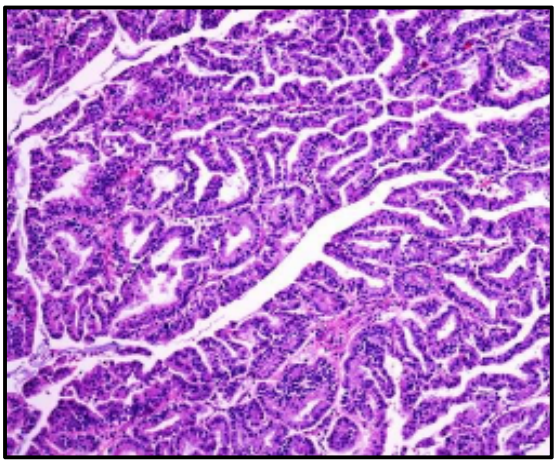


Figure 37 : Tumour cells in papillary pattern with infiltration (100X) HPE- 3469/13

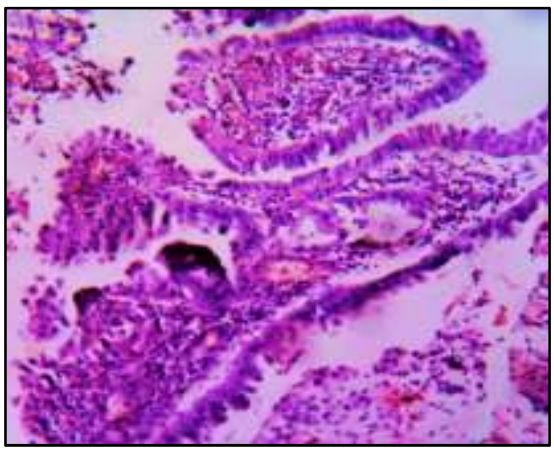


Figure 38 : Cells in delicate papillary pattern with fibro – vascular core (400X) HPE – 3469/13

MUCINOUS ADENOCARCINOMA

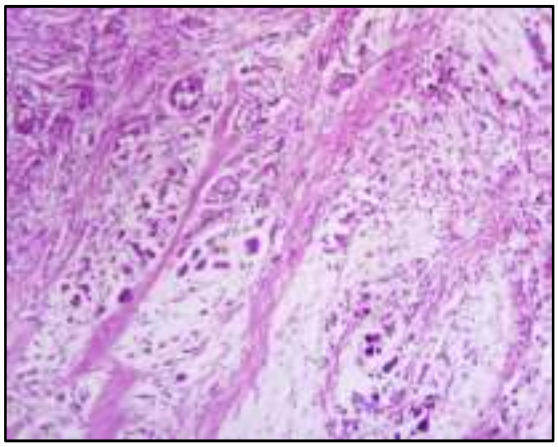


Figure 39 : Chains and clusters of malignant cells floating in extra – cellular mucin pool (100X) HPE – 1933/13

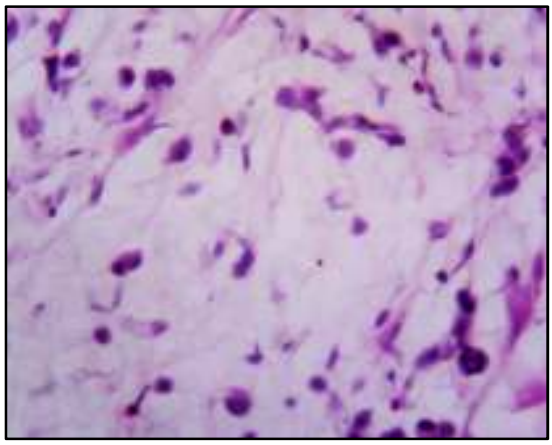


Figure 40 : Malignant epithelial cells with pleomorphism and scattered signet ring cells (400X) HPE – 1933/13

SIGNET RING ADENOCARCINOMA

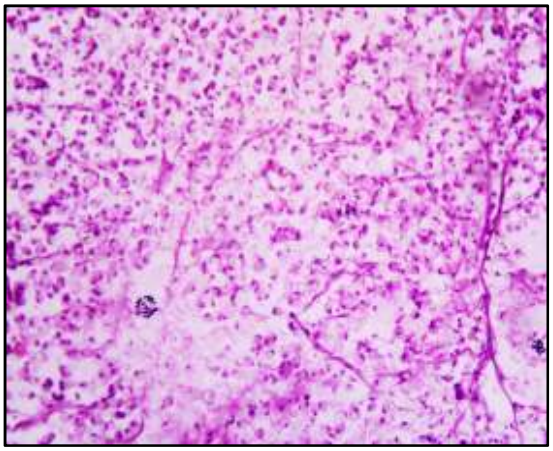


Figure 41 : Sheets of signet ring cells forming >50% of the tumour. (100X) HPE – 3129/13

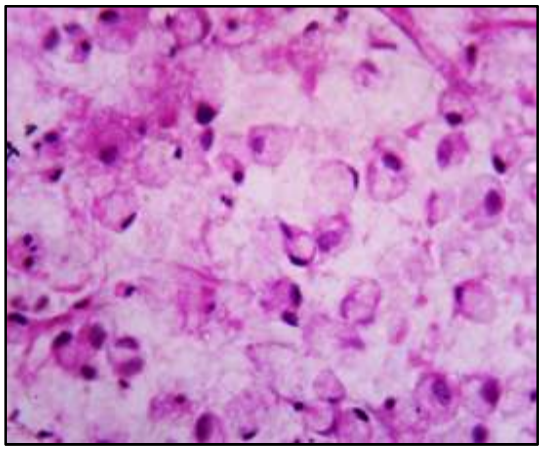


Figure 42 : Sheets of malignant cells with abundant intra-cytoplasmic mucin. (400X) HPE – 3129/13

OTHER PROGNOSTIC FACTORS

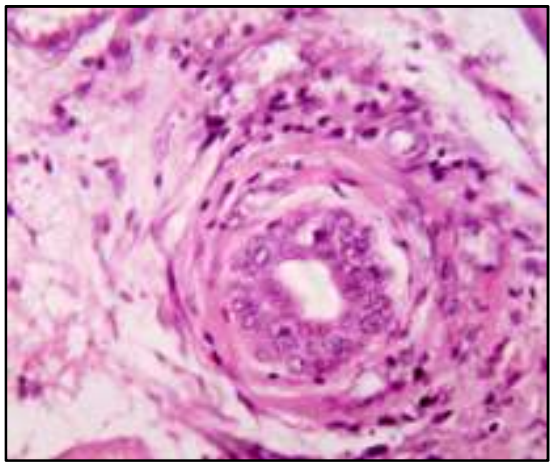


Figure 43 : Vascular invasion (400X)

HPE – 3129/13

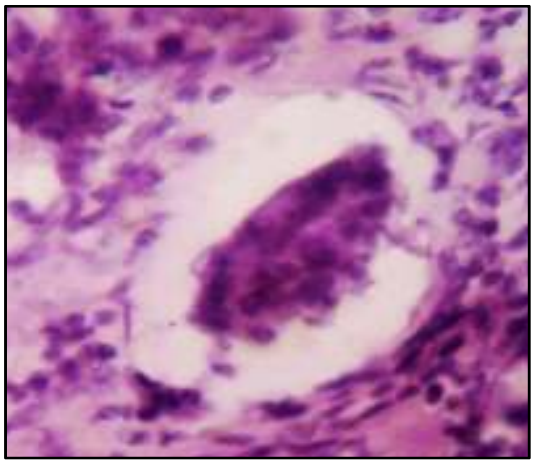


Figure 44 : Lymphatic invasion (400X)

HPE - 2704/12

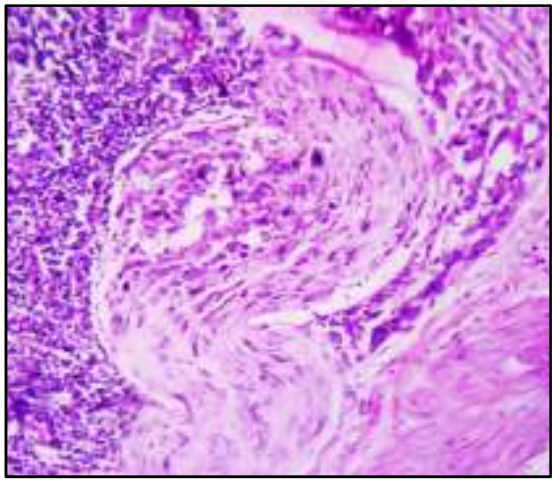


Figure 45 : Perineural infiltration (100X)

HPE – 1069/14 (100X)

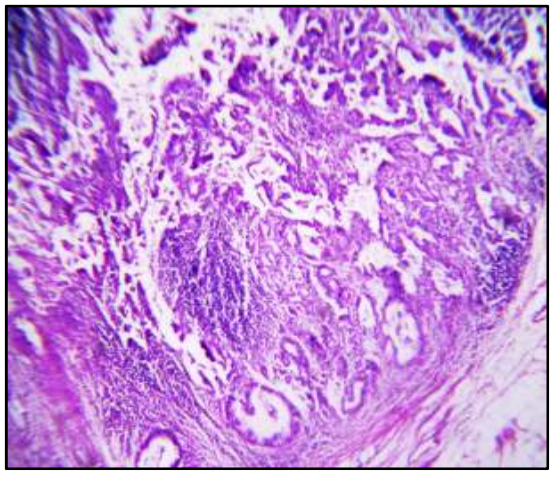


Figure 46 : Metastatic deposit in node

HPE – 2271/13

HER2/neu EXPRESSION

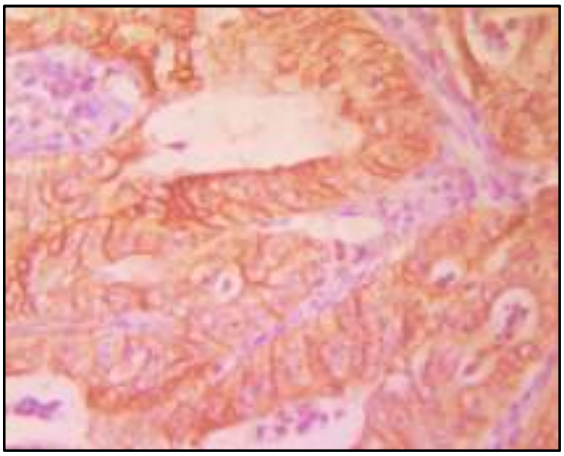


Figure 47: Membranous HER2/neu 3+ positivity HPE-2398/12

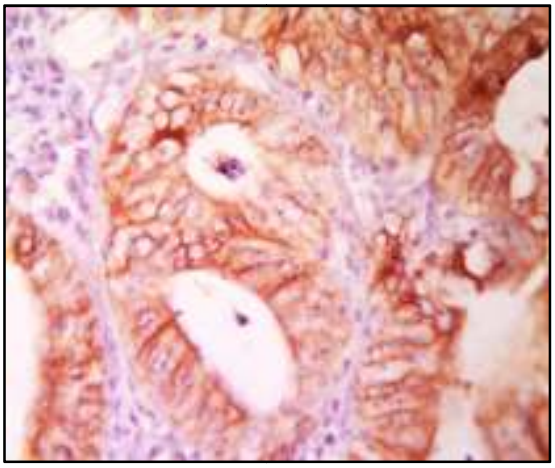


Figure 48 : HER2 neu membranous positivity (3+) HPE-1195/14

BIBILIOGRAPHY:

1. Hamilton, S. R. and Alton, L. (eds.) (2000) Pathology and Genetics of Tumours of the Digestive System. IARC Press: Lyon (2000) Pathology and Genetics. Tumours of the Digestive System. WHO Classification of Tumours, Volume 2. IARC Press: Lyon
2. Ferlay J, Shin HR, Bray F, Forman D, Mathers C, Parkin DM. GLOBOCAN 2008, Cancer incidence and mortality worldwide: IARC Cancer Base No. 10 [Internet]. Lyon (France): IARC; 2010, Available from: http://globocan.iarc.fr.
3. Catalano V, Labianca R, Beretta GD, Gatta G, de Braud F, Van Cutsem E. Gastric cancer. Crit Rev Oncol Hematol 2009; 71: 127-164
4. Danaei G, Vander Hoorn S, Lopez AD, Murray CJ, Ezzati M. Causes of cancer in the world: comparative risk assessment of nine behavioural and environmental risk factors. Lancet 2005; 366: 1784-1793
5. Jemal A, Bray F, Center MM, Ferlay J, Ward E, Forman D. Global cancer statistics. CA Cancer J Clin.2011;61:69–90. [PubMed]
6. Schwartz G. Invasion and metastasis in gastric cancer: in vitro and in vivo models with clinical considerations. Semin Oncol. 1996;23:316– 324
7. Howe HL, Wu X, Ries LA, Cokkinides V, Ahmed F, Jemal A, Miller B, Williams M, Ward E, Wingo PA, et al. Annual report to the nation on the status of cancer, 1975-2003, featuring cancer among U.S. Hispanic/Latino populations. Cancer. 2006;107:1711–1742. [PubMed]

8. Goldstein DB, Hirschhorn JN. In genetic control of disease, does 'race' matter. Nat Genet. 2004;36:1243–1244. [PubMed]
9. Jemal A, Siegel R, Ward E, Murray T, Xu J, Thun MJ. Cancer statistics, 2007. CA Cancer J Clin.2007;57:43–66. [PubMed]
10. Henson DE, Dittus C, Younes M, Nguyen H, bores-Saavedra J (2004) Differential trends in the intestinal and diffuse types of gastric carcinoma in the United States, 1973-2000: increase in the signet ring cell type. Arch Pathol Lab Med 128, 765-770.
11. Indian Council of Medical Research (ICMR), First Report of the Population Based Cancer Registries Under North Eastern Regional Cancer Registry 2003-2004. [last cited on 2010 Sep 6]. Available from: http://www.icmr.nic.in/ncrp/first_report_2003-04/first_report.htm
12. Cunningham D, Allum WH, Stenning SP et al. Perioperative chemotherapy versus surgery alone for resectable gastroesophageal cancer. New Eng J Med 2006; 355(1): 11–22
13. A. Ohtsu, "Chemotherapy for metastatic gastric cancer: past, present, and future," Journal of Gastroenterology, vol. 43, no. 4, pp. 256–264, 2008.
14. Jain VK, Cunningham D, Chau I. Preoperative and postoperative chemotherapy for gastric cancer. Surgical oncology clinics of North America. 2012 Jan 2012;21(1):99-112.
15. Gravalos C, Jimeno A. HER2 in gastric cancer: a new prognostic factor and a novel therapeutic target. Annals of oncology : official journal of the

European Society for Medical Oncology / ESMO. 2008 Sep 2008;19(9):1523-1529.

16. Allgayer H, Babic R, Gruetzner KU, Tarabichi A, Schildberg FW, Heiss MM. c-erbB-2 is of independent prognostic relevance in gastric cancer and is associated with the expression of tumor-associated protease systems. Journal of clinical oncology : official journal of the American Society of Clinical Oncology. 2000 Jun 2000;18(11):2201-2209
17. De Vita F, Giuliani F, Silvestris N, Catalano G, Ciardiello F, Orditura M. Human epidermal growth factor receptor 2 (HER2) in gastric cancer: a new therapeutic target. Cancer treatment reviews. 2010 Nov 2010;36S3:S11-S15.
18. Boku N. HER2-positive gastric cancer. Gastric cancer : official journal of the International Gastric Cancer Association and the Japanese Gastric Cancer Association. 2013 Apr 7 2013
19. Hechtman JF, Polydorides AD. HER2/neu gene amplification and protein overexpression in gastric and gastroesophageal junction adenocarcinoma: a review of histopathology, diagnostic testing, and clinical implications. Archives of pathology & laboratory medicine. 2012 Jun 2012;136(6):691-697.
20. Langman's Medical Embryology (11th.ed.):ch. 14,pp. 209-233
21. Grand RJ, Watkins JB, Torti FM. Development of the human gastrointestinal tract. Areview. Gastroenterology, 1976; 70: 790

22. Lymphatic drainage of the stomach. (From Moody F, McGreevy J, Miller T: Stomach. In Schwartz SI, Shires GT [eds]: Principles of Surgery, 5th ed. New York, McGraw-Hill, 1989.)
23. From Mercer D, Liu T: Open truncal vagotomy. In Operative Techniques in General Surgery 5:8-85, 2003
24. Smith B. The Neuropathology of the Alimentary Tract. London: Edward Arnold, 1972.
25. Day DW, Morson BC. Structure and infrastructure. Gastrointest Res, 1980; 6: 1
26. Rubin W, Ross LL, Sleisenger MH, Jeffries GH. The normal human gastric epithelia. Afine structural study. Lab Invest, 1968; 19: 598.
27. Pavithran K, Doval DC, Pandey KK. Gastric cancer in India. Gastric laser Cancer. 2002;5:240–3[PubMed]
28. Yeole BB. Trends in cancer incidence in esophagus, stomach, colon, rectum and liver in males in India. Asian Pac J Cancer Prev. 2008;9:97–100. [PubMed].
29. Satyanarayana L, Asthana S. Life time risk for development of ten major cancers in India and its trends over the years 1982 to 2000. Indian J Med Sci. 2008;62:35–44. [PubMed]
30. Rastogi T, Devesa S, Mangtani P, Mathew A, Cooper N, Kao R, et al. Cancer incidence rates among South Asians in four geographic regions: India, Singapore, UK and US. Int J Epidemiol.2008;37:147–60. [PubMed]

31. Shinkan Tokudome. Gastric Cancer. In: Tuncer AM, editor. Asian Pacific organization for cancer prevention: cancer report, 2010. Ankara, Turkey: Asian Pacific organization for cancer prevention. Suvak, New hope in health foundation; 2010.
32. Dikshit R, Gupta PC, Ramasundarahettige C, Gajalakshmi V, Aleksandrowicz L, Badwe R, Kumar R, Jha P. Cancer mortality in India: a nationally representative survey. Lancet 2012; 379: 1807-1816
33. Gore R. Gastrointestinal cancer. Radiol Clin North Am. 1997;35:295– 310.
34. Phukan RK, Zomawia E, Hazarika NC, Baruah D, Mahanta J. High prevalence of stomach cancer among the people of Mizoram, India. Curr Sci. 2004;87:285–6.
35. Time trends in Cancer Incidence rates 1982-2005. Indian council of medical research; 2009. A report by National Cancer registry Programme
36. correa P. A human model of gastric carcinogenesis. Cancer Res 1988;48:3554-66.
37. Marshall BJ, Warren JR. Unidentified curved bacilli in the stomach of patients with gastritis and peptic ulceration. Lancet. 1984;1(8390):1311-5.
38. Catalano V, Labianca R, Beretta GD, Gatta G, de Braud F, Van Cutsem E. Gastric cancer. Crit Rev Oncol Hematol 2009; 71: 127-164
39. Houghton J, Wang TC. Helicobacter pylori and gastric cancer: a new paradigm for inflammation-associated epithelial cancers. Gastroenterology 2005; 128: 1567-1578

40. Graham, D. Y., Malaty, H. M., Evans, D. G., Evans, D. J., Klein, P. D., & Adam, E. (1991). Epidemiology of Helicobacter pylori in an asymptomatic population in the United States. Gastroenterology, *100*(1495), 501.
41. Fock KM, Ang TL. Epidemiology of Helicobacter pylori infection and gastric cancer in Asia. J Gastroenterol Hepatol 2010; 25: 479-486
42. Cover TL and Blaser MJ (1995) Helicobacter pylori: a bacterial cause of gastritis, peptic ulcer disease, gastric cancer. Am Soc Microbiol News 61, 21-26.
43. Peek RM Jr, Crabtree JE: Helicobacter infection and gastricneoplasia. J Pathol 2006;208:233–248
44. Brenner H, Arndt V, Bode G, Stegmaier C, Ziegler H, Stumer T. Risk of gastric cancer among smokers infected with Helicobacter pylori. Int J Cancer. 2002;98(3):446-9.
45. Wu AH, Crabtree JE, Bernstein L, Hawtin P, Cockburn M, Tseng CC, Forman D. Role of Helicobacter pylori CagA+ strains and risk of adenocarcinoma of the stomach and esophagus. Int J Cancer. 2003;103(6):815-21.
46. Hamajima N, Goto Y, Nishio K, Tanaka D, Kawai S, Sakakibara H, Kondo T. Helicobacter pylori eradication as a preventive tool against gastric cancer. Asian Pac J Cancer Prev. 2004;5(3):246-52.

47. Sauvaget C, Lagarde F, Nagano J, Soda M, Koyama K, Kodama K. Lifestyle factors, radiation and gastric cancer in atomic-bomb survivors (Japan). Cancer Causes Control. 2005;16(7):773-80.

48. Sauvaget C, Nagano J, Hayashi M, Spencer E, Shimizu Y, Allen N. Vegetables and fruit intake and cancer mortality in the Hiroshima/Nagasaki Life Span Study. Br J Cancer. 2003;88(5):689-94.

49. Engel LS, Chow WH, Vaughan TL, Gammon MD, Rotterdam H, West AB, Blaser M, Blot WJ, Gail MH, Fraumeni JF Jr. Population attributable risks of esophageal and gastric cancers. J Natl Cancer Inst. 2003;95(18):1404-13.

50. Lindblad M, Rodriguez LA, Lagergren J. Body mass, tobacco and alcohol and risk of esophageal, gastric cardia, and gastric non-cardia adenocarcinoma among men and women in a nested case-control study. Cancer Causes Control. 2005;16(3):285-94.

51. Ji BT, Chow WH, Yang G, McLaughlin JK, Gao RN, , Gao YT. The influence of cigarette smoking, alcohol, and green tea consumption on the risk of carcinoma of the cardia and distal stomach in Shanghai, China. Cancer. 1996;77(12):2449-57.

52. El-Omar EM, Carrington M, Chow WH, McMoll KE, Bream JH, Young HA, et al. Interleukin-1 polymorphism associated with increased risk of gastric cancer. Nature. 2000;404:398–402.[PubMed]

53. Yamagata H, Kiyohara Y, Nakamura S, Kubo M, Tanizaki Y, Matsumoto T, Tanaka K, Kato I, Shirota T, Iida M. Impact of fasting plasma glucose

levels on gastric cancer incidence in a general Japanese population: the Hisayama study. Diabetes Care. 2005;28(4):789-94.

54. Jenab M, Riboli E, Ferrari P, Sabate J, Slimani N, Norat T, Gonzalez CA: Plasma and dietary vitaminC levels and risk of gastric cancer in the European Prospective Investigation into Cancer and Nutrition (EPIC-EURGAST). Carcinogenesis. 2006;27:2250–2257)

55. Correa P, Fontham ET, Bravo JC, Bravo LE, Ruiz B, Zarama G, Realpe JL, Malcom GT, Li D, Johnson WD, Mera R: Chemoprevention of gastric dysplasia: randomized trial of antioxidant supplements and anti-Heli.

56. Fujino Y, Tamakoshi A, Ohno Y, Mizoue T, Tokui N, Yoshimura T; JACC Study Group. Japan Collaborative Cohort Study for Evaluation of Cancer Risk. Prospective study of educational background and stomach cancer in Japan. Prev Med. 2002;35(2):121-7.

57. Japanese classification of gastric carcinoma: 3rd English edition. Gastric Cancer, 2011.14(2): p. 101-12

58. Brenner H, Arndt V, Sturmer T, Stegmaier C, Ziegler H, Dhom G. Individual and joint contribution of family history and Helicobacter pylori infection to the risk of gastric carcinoma. Cancer. 2000;88(2): 274-9

59. Uemura N, Okamoto S, Yamamoto S, Matsumura N, Yamaguchi S, Yamakido M, Taniyama K, Sasaki N, Schlemper RJ (2001) Helicobacter pylori infection and the development of gastric cancer. N Engl J Med 345, 784-789.

60. Huntsman DG, Carneiro F, Lewis FR, MacLeod PM, Hayashi A, Monaghan KG, Maung R, Seruca R, Jackson CE, Caldas C (2001) Early gastric cancer in young, asymptomatic carriers of germ-line E-cadherin mutations. N Engl J Med 344, 1904-1909

61. Caldas C, Carneiro F, Lynch HT, Yokota J, Wiesner GL, Powell SM, Lewis FR, Huntsman DG, Pharoah PD, Jankowski JA, Jackson CE (1999) Familial gastric cancer: overview and guidelines for management. J Med Genet 36, 873-880

62. Gayther SA, Gorringe KL, Ramus SJ, Huntsman D, Roviello F, Grehan N, Machado JC, Pinto E, Seruca R, Halling K, MacLeod P, Caldas C (1998) Identification of germ-line E-cadherin mutations in gastric cancer families of European origin. Cancer Res 58, 4086-4089.

63. Stone AL, Kroeger M, Sang QX (1999) Structure-functionanalysis of the ADAM family of disintegrin-like andmetalloproteinase-containing proteins. J Protein Chem 18, 447-465.

64. .Erickson HP. Tenascin-C, tenascin-R and tenascin-X: a family of talented proteins in search of functions. Curr Opin Cell Biol. 1993;5; 869-76.

65. Caygill CP, Hill MJ, Kirkham JS, Northfield TC (1986) Mortality from gastric cancer following gastric surgery for peptic ulcer. Lancet 1, 929-931.

66. Grad A (1984) Carcinoma of the resected stomach. In: Ming SC (eds.) Precursors of gastric cancer. Praeger: Philadelphia

67. Magnus HA. Are-assessment of the gastric lesion in pernicious anaemia. J Clin Pathol, 1958; 11: 289.

68. Ye W, Nyren O. Risk of cancers of the oesophagus and stomach by histology or subsite in patients hospitalised for pernicious anaemia. Gut. 2003;52(7):938-41.

69. Shibata D, Weiss LM. Epstein-Barr virus-associated gastric adenocarcinoma. Am J Pathol. 1992;140(4):769-74.

70. Fenogilo-Preiser C, Carneiro F, Correa P, et al. Gastric carcinoma. In: Hamilton S, Aaltonin L, eds. Pathology and Genetics. Tumors of theDigestive System, vol 1. Lyon, France: Lyon Press; 2000:37–52.

71. Albert C. Clinical Aspects of Gastric Cancer. Gastrointestinal Cancers: Biology, Diagnosis, and Therapy. Philadelphia: Lippincot-Raven;1995:197–216.

72. Ming, Si-Chun. "Gastric carcinoma: a pathobiological classification." Cancer39.6 (1977): 2475-2485.

73. Carneiro F (1997). The distinction between dysplasia and truly invasive cancer. Classification of gastric carcinomas. Curr Diagn Patho l 4: 51-59.

74. Lauren P. The two histological main types of gastric carcinoma: diffuse and so-called intestinal-type carcinoma. An attempt at a histo-clinical classification. Acta Pathol Microbiol Scand 1965; 64: 31–49.

75. Nakamura K, Sugano H, Takagi K. Carcinoma of the stomach in incipient phase: its histogenesis and histological appearances. Gann 1968; 59(3): 251–8.

76. Kabashima A, Yao T, Sugimachi K, Tsuneyoshi M. Relationship between biologic behavior and phenotypic expression in intramucosal gastric carcinomas. Hum Pathol 2002; 33(1): 80–6.

77. Sugihara H, Hattori T, Imamura Y, Noriki S, Fukuda M, Katsura K, et al. Morphology and modes of cell proliferation in earliestsignet-ring-cell carcinomas induced in canine stomachs by Nethyl-N'-nitro-N-nitrosoguanidine. J Cancer Res Clin Oncol1991; 117(3): 197–204.

78. Saito K, Shimoda T. The histogenesis and early invasion of gastric cancer. Acta Pathol Jpn 1986; 36(9): 1307–18.

79. Tian MM, Zhao AL, Li ZW, Li JY. Phenotypic classification of gastric signet ring cell carcinoma and its relationship with clinicopathologic parameters and prognosis. World J Gastroenterol 2007; 13(23): 3189–98.

80. Tatematsu M, Tsukamoto T, Inada K. Stem cells and gastric cancer: role of gastric and intestinal mixed intestinal metaplasia. Cancer Sci 2003; 94(2): 135–41.

81. Koseki K, Takizawa T, Koike M, Ito M, Nihei Z, Sugihara K. Distinction of differentiated type early gastric carcinoma with gastric type mucin expression. Cancer 2000; 89(4): 724–32.

82. Morohara K, Tajima Y, Nakao K, Nishino N, Aoki S, Kato M, et al. Gastric and intestinal phenotypic cell marker expressions in gastric differentiated-type carcinomas: association with Ecadherin expression and chromosomal changes. J Cancer Res Clin Oncol 2006; 132(6): 363–75.

83. Akamatsu T, Katsuyama T. Histochemical demonstration of mucins in the intramucosal laminated structure of human gastric signet ring cell carcinoma and its relation to submucosal invasion. Histochem J 1990; 22(8): 416–25.

84. Nguyen MD, Plasil B, Wen P, Frankel WL. Mucin profiles in signet- ring cell carcinoma. Arch Pathol Lab Med 2006; 130(6): 799–804.

85. Jass JR, Walsh MD. Altered mucin expression in the gastrointestinal tract: a review. J Cell Mol Med 2001; 5(3): 327–51.

86. Lauwers GY, Carneiro F, Graham DY. Gastric carcinoma. In: Bowman FT, Carneiro F, Hruban RH, eds. Classification of Tumours of the Digestive System. Lyon:IARC;2010. In press.

87. Endoh Y, Tamura G, Motoyama T, Ajioka Y, Watanabe H (1999). Well-differentiated adenocarcinoma mimicking complete- type intestinal metaplasia in the stomach. Hum Pathol 30: 826-832.

88. Nishikura K, Watanabe H (1997). Gastric microcarcinoma. Its histopathological characteristics. In: Progress in Gastric Cancer Research 1997, Siewert JR, Roder JD (eds), Monduzzi Editore: Bologna, Italy

89. Wu MS, Shun CT, Wu CC, et al. Epstein-Barr virus associated gastric carcinomas: relation to H. pylori infection and genetic alterations. Gastroenterology 2000;118:1031-8.
90. Wang HH, Wu MS, Shun CT, et al. Lymphoepitheliomalike carcinoma of the stomach: a subset of gastric carcinoma with distinct clinicopathological features and high prevalence of Epstein-Barr virus infection. Hepatogastroenterology 1999;46:1214-9.
91. Fu DX, Tanhehco Y, Chen J, et al. Bortezomib-induced enzyme-targeted radiation therapy in herpes virus associated tumors. Nat Med 2008;14:1118-22.
92. Halling KC, Harper J, Moskaluk CA, et al. Origin of microsatellite instability in gastric cancer. Am J Pathol 1999;155:205-11.
93. Wu CW, Chen GD, Jiang KC, et al. A genome-wide study of microsatellite instability in advanced gastric carcinoma. Cancer 2001;92:92-101.
94. Thibodeau SN, French AJ, Roche PC, et al. Altered expression of hMSH2 and hMLH1 in tumors with microsatellite instability and genetic alterations in mismatch repair genes. Cancer Res 1996;56:4836-40.
95. Grogg KL, Lohse CM, Pankratz VS, et al. Lymphocyterich gastric cancer: associations with Epstein-Barr virus, microsatellite instability, histology, and survival. Mod Pathol 2003;16:641-51.

96. Spigelman AD, Williams CB, Talbot IC, Domizio P, Phillips RK. Upper gastrointestinal cancer in patients with familial adenomatous polyposis. Lancet. 1989;2(8666):783-5.

97. Daibo M, Itabashi M, Hirota T. Malignant transformation of gastric hyperplastic polyps. Am J Gastroenterol. 1987;82(10):1016-25.

98. IARC (1994) proceedings of the IARC working group on the evaluation of carcinogenic risks to humans. schistosomes,liver flukes and helicobacter pylori. IARC Monogr EvalCarcinog Risks Hum 61:1-241., 1-241.

99. Larsson SC, Giovannucci E, Wolk A (2006) Folate intake, MTHFR polymorphisms, risk of esophageal, gastric, pancreatic cancer: a meta-analysis. Gastroebterology 131,1271-1283.

100. Kodama Y, Inokuchi K, Soejima K, Matsusaka T, Okamura T (1983). Growth patterns and prognosis in early gastric carcinoma. Superficially spreading and penetrating growth types. Cancer 51: 320-326.

101. Wang, Y. Y., Ye, Z. Y., Li, L., Zhao, Z. S., Shao, Q. S., & Tao, H. Q. (2011). ADAM 10 is associated with gastric cancer progression and prognosis of patients. Journal of surgical oncology, 103(2), 116-123.

102. Yu, G. Z., Chen, Y., & Wang, J. J. (2009). Overexpression of Grb2/HER2 signaling in Chinese gastric cancer: their relationship with clinicopathological parameters and prognostic significance. Journal of cancer research and clinical oncology, 135(10), 1331-1339

103. Ebert, M. P, Fei, G, Kahmann, S, Müller, O, Yu, J, Sung, J. J, & Malfertheiner, P. Increasedbeta-catenin mRNA levels and mutational alterations of the APC and betacateningene are present in intestinal-type gastric cancer. Carcinogenesis (2002). , 23, 87-91.

104. Lehnert T, Erlandson RA, Decosse JJ: Lymph and blood capillaries of the human gastric mucosa. A morphologic basis for metastasis in early gastric carcinoma. Gastroenterology 1985; 89:939-950.

105. Wu CW, Hsieh MJ, Lo SS, Tsay SH, Lui WY, P'eng FK: Lymph node metastasis from carcinoma of the distal one-third of the stomach. Cancer 1994; 73:3109-3114.

106. Fang, D. C, Luo, Y. H, Yang, S. M, Li, X. A, Ling, X. L, & Fang, L. Mutation analysis of APC gene in gastric cancer with microsatellite instability. World Journal of Gastroenterolology(2002). , 8, 787-791.

107. Oliveira, C, Senz, J, Kaurah, P, Pinheiro, H, Sanges, R, Haegert, A, Corso, G, Schouten,J, Fitzgerald, R, S, Huntsman,D. Germline CDH1 deletions in hereditary diffuse gastric cancer families. Human Molecular Genetics (2009). , 18, 1545-1555.

108. Tahara E (2004) Genetic pathways of two types of gastric cancer. IARC Sci Publ, 327-349.

109. Vogelstein B, Fearon ER, Hamilton SR, Kern SE, Preisinger AC, Leppert M, Nakamura Y, White R, Smits AM, Bos JL (1988) Genetic alterations during colorectal-tumor development. NEngl J Med 319, 525-532.

110. Feng CW, Wang LD, Jiao LH, Liu B, Zheng S, Xie XJ (2002) Expression of p53, inducible nitric oxide synthase and vascular endothelial growth factor in gastric precancerous and cancerous lesions: correlation with clinical features. BMC Cancer 2, 8.

111. Mattioli E, Vogiatzi P, Sun A, Abbadessa G,Gaughan JP, Claudio PP (2007) Immunohistochemical analysis of pRb2/p130, VEGF, EZH2, p53, p16(INK4A), p27(KIP1), p21(WAF1), Ki-67 expression patterns in gastric cancer. J Cell Physiol 210, 183-191.

112. Pinto M, Oliveira C, Cirnes L, Carlos MJ, Ramires M, Nogueira A, Carneiro F, Seruca R (2003) Promoter methylation of TGFbeta receptor I and mutation of TGFbeta receptor II are frequent events in MSI sporadic gastric carcinomas. J Pathol 200, 32-38.

113. Nakatsuru S, Yanagisawa A, Ichii S, Tahara E, Kato Y, Nakamura Y, Horii A. Somatic mutation of the APC gene in gastric cancer: frequent mutations in very well differentiated adenocarcinoma and signet-ring cell carcinoma. Hum MolGenet 1992; 1: 559-563.

114. Ma YY, Tao HQ. Microribonucleic acids and gastric cancer. Cancer Sci 2012; 103: 620-625.

115. Park WS, Oh RR, Park JY, Lee SH, Shin MS, Kim YS, Kim SY, Lee HK, Kim PJ, Oh ST, Yoo NJ, Lee JY (1999) Frequent somatic mutations of the beta-catenin gene in intestinal-type gastric cancer. Cancer Res 59, 4257-4260.

116. Hudler P. Genetic aspects of gastric cancer instability. ScientificWorldJournal 2012; 2012: 761909.

117. Park SY, Kook MC, Kim YW, Cho NY, Jung N, Kwon HJ, Kim TY, Kang GH. CpG island hypermethylator phenotype in gastric carcinoma and its clinicopathological features. VirchowsArch 2010; 457: 415-422

118. Mitani Y, Oue N, Hamai Y, Aung PP, Matsumura S, Nakayama H, et al. Histone H3 acetylation is associated with reduced p21WAF1/CIP1 expression in gastric carcinoma. J Pathol 2005;205:65–73.

119. Hermanek P, Sobin LH, editors. TNM classification of malignant tumours. Fourth Edition. Berlin: Springer-Verlag, 1987.

120. Kim JP, Lee JH, Kim SJ, Yu HJ, Yang HK. Clinicopathologic characteristics and prognostic factors in 10 783 patients with gastric cancer. Gastric Cancer. 1998;1(2):125-133.

121. Bonenkamp JJ, Hermans J, Sasako M, van de Velde CJ: Extended lymph-node dissection for gastric cancer. Dutch Gastric Cancer Group. N Engl J Med 1999; 340:908-914.

122. D′souza MA, Singh K, Shrikhande SV. Surgery for gastric cancer: An evidence-based perspective. J Cancer Res Ther. 2009;5:225–31. PubMed

123. Paoletti X, Oba K, Burzykowski T, Buyse MGASTRIC (Global Advanced/Adjuvant stomach Tumor Research International Collaboration) Group: Benefit of adjuvant chemotherapy for resectable gastric cancer: a metaanalysis JAMA 2010; 303:1729-1737.

124. Imanaga H, Nakazato H: Results of surgery for gastric cancer and effect of adjuvant mitomycin C on cancer recurrence. World J Surg 1977; 1:213-220.

125. Shah MA, Kelsen DP: Gastric cancer: a primer on the epidemiology and biology of the disease and an overview of the medical management of advanced disease. J Natl Compr Canc Netw 2010; 8:437-447

126. Yokota T, Ishiyama S, Saito T, Teshima S, Narushima Y, Murata K, Iwamoto K, Yashima R, Yamauchi H, Kikuchi S (2004) Lymph node metastasis as a significant prognostic factor in gastric cancer: a multiple logistic regression analysis. ScandJ Gastroenterol 39, 380-384

127. Kim MA, Lee HS, Yang HK, Kim WH. Clinicopathologic and protein expression differences between cardia carcinoma and noncardia carcinoma of the stomach. Cancer. 2005;103(7):1439-46. b)

128. Sipponen P, Correa P. Delayed rise in incidence of gastric cancer in females results in unique sex ratio (M/F) pattern: etiologic hypothesis. Gastric Cancer. 2002;5(4):213-9.

129. Fujimoto S, Takahashi M, Ohkubo H, Mutou T, Kure M, Masaoka H, Kobayashi K. Comparative clinicopathologic features of early gastric cancer in young and older patients. Surgery. 1994;115(4):516-20.

130. Kath R, Fiehler J, Schneider CP, Hoffken K. Gastric cancer in very young adults: apropos four patients and a review of the literature. J Cancer Res Clin Oncol. 2000;126(4):233-7.

131. Kokkola A, Sipponen P. Gastric carcinoma in young adults. Hepatogastroenterology. 2001;48(42):1552-5.

132. Blair SL, Schwarz RE. Advanced age does not contribute to increased risks or poor outcome after major abdominal operations. Am Surg. 2001;67(12):1123-7

133. Crawford J. The gastrointestinal tract. In: Robbins SLCR, Kumar V, Schoen FJ, eds. Pathologic Basis of Disease. Philadelphia: WB Saunders Co; 1994:755–783.

134. Takahashi I, Matsusaka T, Onohara T, et al. Clinicopathological features of long-term survivors of scirrhous gastric cancer. Hepatogastroenterology. 2000;47:1485–1488.

135. Kim TH, Han SU, Cho YK, et al. Perigastric lymph node status can be a simple prognostic parameter in patients with gastric cancer. Hepatogastroenterology. 2000;47:1475–1478.

136. Adachi Y, Shiraishi N, Suematsu T, et al. Most important lymph node information in gastric cancer: multivariate prognostic study. Ann SurgOncol. 2000;7:503–507.

137. Msika S, Benhamiche A, Jouve J-L, et al. Prognostic factors after curative resection of gastric cancer. A population-based study. Eur JCancer. 2000;36:390 –396.

138. Yokota T, Kunii Y, Teshima S, et al. Significant prognostic factors in patients with early gastric cancer. Int Surg. 2000;85:286 –290.

139. Borch K, Jonsson B, Tarpila E, et al. Changing patterns of histological type, location, stage, and outcome of surgical treatment of gastric carcinoma. Br J Surg. 2000;87:618–626.

140. Pinto-de-sousa J, David L, Seixas M, et al. Clinicopathologic profiles and prognosis of gastric carcinoma from the cardia, fundus/body and antrum. Dig Surg. 2001;18:102–110.

141. Yokota T, Kunii Y, Teshima S, et al. Significant prognostic factors in patients with node-negative gastric cancer. Int Surg. 1999;84:331–336.

142. Kikuchi S, Sato M, Katada N, et al. Surgical outcome of node-positive early gastric cancer with particular reference to nodal status. AnticancerRes. 2000;20:3695–3700.

143. Maehara Y, Kakeji Y, Koga T, et al. Therapeutic value of lymph node dissection and the clinical outcome for patients with gastric cancer. Surgery. 2002;131(suppl):S85–91

144. Lello E, Furnes B, Edna TH (2007) Short and long-term survival from gastric cancer. A population-based study from a county hospital during 25 years. Acta Oncol 46, 308-315.

145. Tanaka K, Kiyohara Y, Kato I, Matsumoto T, Yamagata H, Kubo M, Tanizaki Y, Okubo K, Nakamura H, Iwamoto H, Nakayama K, Iida M (2004) Incidence and prognosis of gastric cancer in a population-based cohort survey: the Hisayama study. Scand J Gastroenterol 39, 459-463.

146. Marchet A, Mocellin S, Ambrosi A, de MG, Di LA, Marrelli D, Roviello F, Morgagni P, On Behalf Of The Italian Research Group For Gastric Cancer Study Ircg (2007) The prognostic value of N-ratio in patients with gastric cancer: Validation in a large, multicenter series. Eur J Surg Oncol 34,159-65.

147. Borrmann R. Geschwulste des magens und duodenums. En: Henke F, Lanbarsch O, editors. Handbuch der speziellen pathologischen anatomie and histologie. 1st ed. Berlin: Julius Springer, 1926.

148. Chen CY, Wu CW, Lo SS, Hsieh MC, Lui WY, Shen KH. Peritoneal carcinomatosis and lymph node metastasis are prognostic indicators in patients with Borrmann type IV gastric carcinoma. Hepatogastroenterology. 2002;49(45):874-7.

149. Zhang XF, Huang CM, Lu HS, Wu XY, Wang C, Guang GX, Zhang JZ, Zheng CH. Surgical treatment and prognosis of gastric cancer in 2,613 patients. World J Gastroenterol. 2004;10(23):3405-8.

150. Gaspar MJ, Arribas I, Coca MC, et al. Prognostic value of carcinoembryonic antigen, CA 19–9 and CA 72–4 in gastric carcinoma. TumourBiol. 2001;22:318 –322.

151. Starzynska T, Markiewski M, Domagala W, et al. The clinical significance of p53 accumulation in gastric carcinoma. Cancer. 1996;77: 2005–2012.

152. Shimada Y, Yamasaki S, Hashimoto Y, Ito T, Kawamura J, Soma T, Ino Y, Nakanishi Y, Sakamoto M, Hirohashi S, Imamura M (2004) Clinical

significance of dysadherin expression in gastric cancer patients. Clin Cancer Res 10, 2818-2823.

153. Chan AO, Wong BC, Lam SK (2001) Gastric cancer: past, present and future. Can J Gastroenterol 15, 469-474.

154. Stern DF, Heffernan PA, Weinberg RA. p185, a product of the neu proto-oncogene, is a receptorlike protein associated with tyrosine kinase activity. Mol Cell Biol 1986;6:1729–1740.

155. Yasui W, Oue N, Aung PP, Matsumura S, Shutoh M, Nakayama H (2005) Molecular-pathological prognostic factors of gastric cancer: a review. Gastric Cancer 8, 86-94.

156. H. K. W. Koeppen, B. D. Wright, A. D. Burt et al., "Overexpression of HER2/neu in solid tumours: an immunohistochemical survey," Histopathology, vol. 38, no. 2, pp. 96–104, 2001. View at Publisher · View at Google Scholar · View at Scopus

157. S. Kaptain, L. K. Tan, and B. Chen, "Her-2/neu and breast cancer," Diagnostic Molecular Pathology, vol. 10, no. 3, pp. 139–152, 2001. View at Publisher · View at Google Scholar ·View at Scopus

158. Bancroft JD, Marilyn Gamble (Ed), Theory and practice of histological techniques, Churchill Livingstone 2002

159. Pluzek KY, Sweeney E, Miller KD, Isaacson P, A major advance for IHC Epos, J Pathol 169 (Suppl) abstract 220.

160. M. Hofmann, O. Stoss, D. Shi et al., “Assessment of a HER2 scoring system for gastric cancer: results from a validation study,” Histopathology, vol. 52, no. 7, pp. 797–805, 2008.

161. Wolff AC, Hammond ME, Schwartz JN, Hagerty KL, Allred DC, Cote RJ,Wheeler TM, Hayes DF (2007) American Society of Clinical Oncology/College of American Pathologists guideline recommendations for human epidermal growth factor receptor 2 testing in breast cancer. J Clin Oncol 25:118–145

162. Coussens L, Yang-Feng TL, Lluch A et al. Tyrosine kinase receptor with extensive homology to EGF receptor shares chromosomal location with neu oncogene. Science 1985; 230: 1132-9.

163. Slamon DJ, Leyland Jones B, Shak S, Fuchs H, Paton V, Bajamonde A, Fleming T, et al.Use of chemotherapy plus a monoclonal antibody against HER 2 for metastatic breast cancer that over expresses HER2 . N. Engl J. Med. 2001; 344: 783-92.

164. Fukushige S, Matsubara K, Yoshida M, Sasaki M, Yamamoto T. Localization of a novel v-erbB-related gene, c-erbB-2, on human chromosome 17 and its amplification in a gastric cancer cell line. Mol Cell Biol. 1986;6:955–958. [PMC free article] [PubMed]

165. Burstein HJ. The distinctive nature of HER2-positive breastcancers. N Engl J Med 2005; 353: 1652-1654

166. Seshadri R, Firgaira FA, Horsfall DJ, et al. *J Clin Oncol*. 1993;11:1936-1942.Link to abstract on PubMed

167. Grabsch H, Sivakumar S, Gray S, Gabbert HE, Müller W.HER2 expression in gastric cancer: Rare,heterogeneous and of no prognostic value – conclusions from 924 cases of two independent series. Cell Oncol. 2010; 32(1-2):57-65.

168. D. I. Park, J. W. Yun, J. H. Park et al., "HER-2/neu amplification is an independent prognostic factor in gastric cancer," Digestive Diseases and Sciences, vol. 51, no. 8, pp. 1371–1379, 2006. View at Publisher · View at Google Scholar · View at Scopus

169. M. Tateishi, T. Toda, Y. Minamisono, and S. Nagasaki, "Clinicopathological significance of c-erbB-2 protein expression in human gastric carcinoma," Journal of Surgical Oncology, vol. 49, no. 4, pp. 209–212, 1992. View at Publisher · View at Google Scholar · View at Scopus

170. M. Tanner, M. Hollm´en, T. T. Junttila et al., "Amplification of HER-2 in gastric carcinoma: association with topoisomerase IIα gene amplification, intestinal type, poor prognosis and sensitivity to trastuzumab," Annals of Oncology, vol. 16, no. 2, pp. 273–278, 2005.

171. Y. J. Bang, E. VanCutsem, A. Feyereislova et al., "Trastuzumab in combination with chemotherapy versus chemotherapy alone for treatment of HER2-positive advanced gastric or gastro-oesophageal junction cancer

(ToGA): a phase 3, openlabel, randomised controlled trial," The Lancet, vol. 376, no. 9742, pp. 687–697, 2010.

172. A. H. Marx, L. Tharun, J. Muth et al., "HER-2 amplification is highly homogenous in gastric cancer," Human Pathology, vol. 40, no. 6, pp. 769–777, 2009. View at Publisher · View at Google Scholar · View at Scopus

173. Cangiano J, Centeno BA, Garrett CR, et al. Signal transduction proteins in tumors from PuertoRican and Caucasian gastric adenocarcinoma patients: expression differences with potential for specific targeted therapies. Digestive diseases and sciences. 2008 Aug 2008;53(8):2090-2100.

174. Ananiev J, Gulubova M, Manolova I, Tchernev G. Prognostic significance of HER2/neu expression in gastric cancer. Wien Klin Wochenschr 2011; 123: 450-454 [PMID: 21739203 DOI: 10.1007/s00508-011-0025-9]

175. Ghaderi, A., Vasei, M., Maleck-Hosseini, S. A., Gharesi-Fard, B., Khodami, M., Doroudchi, M., & Modjtahedi, H. (2002). The expression of c-erbB-1 and c-erbB-2 in Iranian patients with gastric carcinoma. Pathology Oncology Research, 8(4), 252-256.

176. Halon, A., Donizy, P., Biecek, P., Rudno-Rudzinska, J., Kielan, W., & Matkowski, R. (2012). HER-2 expression in immunohistochemistry has no prognostic significance in gastric cancer patients. The Scientific World Journal,2012

177. . Hofmann, O. Stoss, D. Shi et al., “Assessment of a HER2 scoring system for gastric cancer: results from a validation study,” Histopathology, vol. 52, no. 7, pp. 797–805, 2008.

178. Gravalos C, Jimeno A. HER2 in gastric cancer: a new prognostic factor and a novel therapeutic target. *Ann Oncol* 2008*;* **19***:* 1523–9.

179. Y. Kang, Y. Bang, F. Lordick et al., “Incidence of gastric and gastro-esophageal cancer in the ToGA trial: correlation with HER2 positivity,” in Gastrointestinal Cancer Symposium, vol. 75, abstract 11, 2008

180. Ananiev, J., Gulubova, M., Manolova, I., & Tchernev, G. (2011). Prognostic significance of HER2/neu expression in gastric cancer. Wiener klinische Wochenschrift, 123(13-14), 450-454.

181. Jørgensen JT, Hersom M. HER2 as a Prognostic Marker in Gastric Cancer - A Systematic Analysis of Data from the Literature. J Cancer 2012; 3: 137-144 [PMID: 22481979 DOI: 10.7150/jca.4090

182. Bouché O, Penault-Llorca F. [HER2 and gastric cancer: a novel therapeutic target for trastuzumab]. Bull Cancer 2010; 97: 1429-1440 [PMID: 21134821]

183. Yan SY, Hu Y, Fan JG, Tao GQ, Lu YM, Cai X, Yu BH, Du YQ. Clinicopathologic significance of HER-2/neu protein expression and gene amplification in gastric carcinoma. World J Gastroenterol 2011; 17: 1501-1506 [PMID: 21472111 DOI:10.3748/wjg.v17.i11.1501]

184. Yu, G. Z., Chen, Y., & Wang, J. J. (2009). Overexpression of Grb2/HER2 signaling in Chinese gastric cancer: their relationship with clinicopathological parameters and prognostic significance. Journal of cancer research and clinical oncology, 135(10), 1331-1339.)

ANNEXURE – I

PROFORMA

Case number : Name :

HPE number : Age :

IP number : Gender :

Clinical history :

Risk factors, if any :

Clinical diagnosis :

Imaging :

Endoscopy :

Previous HPE report:

Nature of specimen : Total gastrectomy/Subtotal gastrectomy/Others

GROSS

Proximal circumference : Greater curvature:

Distal circumference : Lesser curvature :

Tumour site :

Tumour size :

Tumour configuration: Depth of invasion:

Margins: Proximal : Distal :

Associated findings :

Total nodes dissected :

MICROSCOPY

Histological type :

Histological grade :

Depth of invasion :

Margins : Proximal : Free / Involved

Distal : Free / Involved

Lymphatic invasion : Present / Absent

Venous invasion : Present / Absent

Perineural invasion : Present / Absent

Associated findings:

Total number of nodes dissected: Number of nodes involved:

Distant metastasis :

TNM staging :

IMMUNOHISTOCHEMISTRY

HER2neu score :

ANNEXURE II

HAEMATOXYLIN & EOSIN STAINING TECHNIQUE[90]

PREPARATION OF HAEMATOXYLIN SOLUTION:

Haematoxylin	2. 5gm
Mercuric oxide	1.25gm
Potassium alum	50gm
Absolute ethyl alcohol	25ml
Sodium iodate	0.5gm
Distilled water	500ml

PROCEDURE:

Potassium alum, 50gm is dissolved in 500ml of distilled water by heating and shaking at 60^0 C. Add solution of 2.5gm of Haematoxylin in 25ml of absolute ethyl alcohol and bring rapidly to boil. When it begins to boil, remove from flame and add 1.25gm of mercuric oxide or sodium iodate. Mix by swirling gently.

PREPARATION OF EOSIN SOLUTION:

Eosin Y	1gm
95% Ethanol	80ml
Glacial Acetic acid	0.2ml

Distilled water 20ml

PROCEDURE:

Dissolve 1gm Eosin Y in 20ml of distilled water and add 80ml of 95% etanol and 0.2ml of glacial acetic acid.

STAINING PROCEDURE:

1.Xylene 3 changes-2mins each.

2.90%, 80%, 70% alcohol-10 dips each.

3. Bring sections to water.

4. Harris Haematoxylin-15 minutes.

5. Rinse in tap water.

6. Differentiate in 1% acid alcohol.

7. Rinse in tap water.

8. Lithium carbonate 0.5%- until blue.

9. Tap water wash.

ANNEXURE III

TNM STAGING OF GASTRIC TUMOURS

T – Primary Tumour

TX - Primary tumour cannot be assessed

T0 - No evidence of primary tumour

Tis - Carcinoma in situ

T1 - Tumour invades lamina propria or submucosa

T2 - Tumour invades muscularispropria

T3 - Tumour penetrates subserosaconnective tissue without invasion of visceral peritoneum or adjacent structures

T4 - Tumour invades serosa oradjacent structures

T4a-Tumour invade serosa

T4b-Tumour invades adjacent structures

N – Regional Lymph Nodes

NX - Regional lymph nodes cannot be assessed

N0 - No regional lymph node metastasis

N1 - Metastasis in 1 or 2 positive lymph nodes

N2 - Metastasis in 3 to 6 positive lymph nodes

N3 - Metastasis in 7 or morepositive lymph nodes

M – Distant Metastasis

MX - Distant metastasis cannot be assessed

M0 - No distant metastasis

M1 - Distant metastasis, positive peritoneal cytology

STAGING

STAGE	T	N	M
Stage 0	Tis	N0	M0
Stage IA	T1	N0	MO
Stage IB	T1	N1	M0
	T2	N0	M0
Stage IIA	T1	N3	M0
	T2	N2	M0
	T3	N1	M0
Stage I I B	T1	N3	M0
	T2	N2	M0
	T3	N1	M0
	T4a	N0	M0
Stage IIIA	T2	N3	M0
	T3	N2	M0
	T4a	N1	M0
Stage IIIB	T3b	N3	M0
	T4a	N2	M0
	T4b	N0/N1	M0
Stage IIIC	T4a	N3	M0
	T4b	N2/N3	M0
Stage IV	Any T	Any N	M1

ANNEXURE IV

PROCESSING FOR IMMUNOHISTOCHEMISTRY

- 3µm thick sections obtained from paraffin embedded blocks and sections taken on poly L- lysine coated adhesive slides . The slides are incubated at 60º C for one hour.
- The slides are subjected to 2 changes of xylene 5 minutes each for deparaffinization.
- They are then transferred to absolute alcohol for 5 minutes followed by by 80% and 70% alcohol for 5 minutes to rehydrate the tissue sections.
- Tissue sections are then placed in running tap water for 5 minutes and washed in distilled water
- Antigen retrieval was performed using pressure cooker in specific buffer (citrate buffer for HER2/neu , Ph 6.0)
- Then the sections are cooled to room temperature and the slides are washed with distilled water.
- 3% Peroxide block was applied for 5 min and washed with a TRIS wash buffer solution for 2 × 5 min to reduce the nonspecific staining due to endogenous peroxidase activity.
- Then protein block was applied and incubated for 5 minutes to block nonspecific background staining.

- Rabbit anti human polyclonal Her2/neu antibody (thermo fisher scicntific) is then added over the tissue and incubated for 30 minutes
- .Followed by the primary horse radish peroxidase polymer amplifier is added for 15 minutes to enhance the process of primary antibody which is then washed in TRIS wash buffer.
- Secondary antibody is added and incubated for 20 minutes and then washed with TRIS wash buffer.
- The bound antibody was visualized using a diaminobenzidine (DAB) chromogen (1ml DAB buffer +1 drop DAB chromogen) and incubate for 5 minutes and then washed with 2 changes of distilled water.
- Counterstaining was done with Mayer's hematoxylin for 30 seconds and washed in tap water.
- Dehydration is done by 2 changes of 100 % alcohol.
- Mounting is done by DPX mountant and observed under microscope.

BUFFER PREPARATIONS

1) Tris – EDTA Buffer: - PH- 9.0

Tris - 6.05 Gm

EDTA - 0.744gms

Distilled water - 1000ml

Section stained by omission of the primary antibody was used as a negative control . Over-expression of HER-2/neu protein in paraffin-embedded invasive breast carcinoma tissue slides was used as a positive control.

case	IP NO	AGE	SEX	PROC	SITE	SIZE	GROSS- PRM	GROSS-DRM	SI	HIST.TYPE	LAUREN'S	T	N	M	STAGE	LI	PNI	MARGINS	RM	TLN	PN	LNR	HER2-neu
18/2012	1005	40	M	P.G	PYLORO-ANTRUM	4×4	NI	I	P	SIGNET	DT	T3	N2	M0	III A	A	A	DISTAL	R2	4	3	0.75	0
418/12	3668	66	M	P.G	BODY	4×2	NI	NI	P	PAPILLARY	IT	T3	N0	M0	II A	P	P	FREE	R0	5	0	0	1+
468/12	5183	40	M	P.G	PYLORO-ANTRUM	2×2	NI	NI	P	TUBULAR	IT	T3	N3	M0	IIIB	A	A	FREE	R0	7	7	1	1+
761/12	13947	37	F	T.G	BODY	10×5	I	I	P	DIFFUSE	DT	T3	N1	M0	IIB	A	A	BOTH	R2	12	2	0.17	0
883/12	18143	50	M	T.G	BODY	7×5	NI	I	A	TUBULAR	IT	T2	N2	M0	IIB	P	A	DISTAL	R2	4	3	0.75	2+
1041/12	19947	62	M	P.G	PYLORO-ANTRUM	5.5×1.5	NI	NI	P	SIGNET	DT	T3	N0	M0	IIA	P	A	FREE	R0	1	0	0	1+
1171/12	22920	42	M	P.G	PYLORO-ANTRUM	3×2	NI	+	P	TUBULAR	IT	T3	N1	M0	IIB	A	A	DISTAL	R2	4	2	0.5	0
1218/12	25442	60	F	P.G	PYLORO-ANTRUM	3×2	NI	NI	P	TUBULAR	IT	T3	N2	M0	III A	P	A	FREE	R0	6	6	1	1+
1497/12	32353	45	F	P.G	PYLORO-ANTRUM	5×3	NI	NI	P	DIFFUSE	DT	T3	N2	M0	III A	P	P	FREE	R0	5	3	0.6	0
1825/12	37677	57	M	P.G	PYLORO-ANTRUM	5×4	NI	I	P	DIFFUSE	DT	T3	N0	M0	IIA	A	A	DISTAL	R2	0	0	0	0
2027/12	43728	57	M	P.G	PYLORUS	4×4	NI	NI	A	PAPILLARY	IT	T2	N2	M0	IIB	A	A	FREE	R0	4	3	0.75	1+
2260/12	45495	65	F	P.G	BODY	4×3	NI	I	A	DIFFUSE	DT	T3	N0	M0	IIA	P	A	DISTAL	R2	6	0	0	0
2289/12	48509	60	M	P.G	PYLORUS	7.5×4	NI	I	P	SIGNET	DT	T3	N1	M0	IIB	A	A	DISTAL	R2	6	2	0.333	0
2300/12	49899	40	M	P.G	PYLORUS	5×4	NI	I	P	TUBULAR	IT	T3	N2	M0	III A	P	P	DISTAL	R2	9	3	0.333	1+
2397/12	47099	45	M	P.G	PYLORUS	7.5×4	NI	I	A	SIGNET	DT	T2	N3	M0	III A	P	A	DISTAL	R2	12	9	0.75	1+
2398/12	48799	51	M	P.G	BODY	2×1	I	NI	A	PAPILLARY	IT	T3	N2	M0	III A	P	P	BOTH	R2	8	4	0.5	3+
2415/12	49474	42	F	P.G	BODY	7×5	I	NI	P	MUCINOUS	DT	T3	N3	M0	IIIB	P	P	BOTH	R2	19	16	0.457	0
2447/12	49640	65	M	P.G	BODY	6×5	I	NI	P	SIGNET	DT	T3	N2	M0	III A	P	P	PROXIMAL	R2	3	3	1	2+
2704/12	55447	49	F	P.G	PYLORO-ANTRUM	3.5×0.7	NI	I	A	SIGNET	IT	T3	N1	M0	IIB	P	P	DISTAL	R2	1	1	1	0
2725/12	55467	58	M	P.G	PYLORO-ANTRUM	6×4	NI	I	A	DIFFUSE	IT	T3	N1	M0	IIB	A	A	DISTAL	R2	4	4	1	0
2763/12	56449	70	F	P.G	PYLORO-ANTRUM	4×4	NI	NI	A	TUBULAR	IT	T2	N0	M0	IB	A	A	FREE	R0	0	0	0	0
2995/12	62956	38	F	P.G	PYLORO-ANTRUM	2.5×2	I	NI	P	PAPILLARY	IT	T3	N1	M0	IIB	A	A	DISTAL	R2	4	2	0.5	0
3095/12	63283	65	M	P.G	PYLORIC ANTRUM	7×4	NI	NI	P	DIFFUSE	DT	T3	N2	M0	IIIA	A	A	FREE	R0	2	1	0.5	1+
3222/12	66480	65	M	P.G	BODY	3.5×2	NI	I	P	TUBULAR	IT	T3	N2	M0	III A	A	A	DISTAL	R2	5	3	0.6	0
3285/12	67363	65	F	P.G	PYLORO-ANTRUM	4×4	NI	I	P	SIGNET	DT	T3	N2	M0	III A	A	A	DISTAL	R2	4	4	1	2+
128/13	2436	65	M	T.G	PYLORO-ANTRUM	4×3	NI	I	A	DIFFUSE	DT	T2	N3	M0	III A	P	A	FREE	R2	11	11	1	0
255/13	5456	55	M	T.G	PYLORO-ANTRUM	7×3	I	NI	P	TUBULAR	IT	T3	N2	M0	III A	P	P	FREE	R2	6	5	0.83	0
1933/13	36456	72	M	P.G	PYLORO-ANTRUM	6×1.5	NI	NI	A	MUCINOUS	DT	T2	N2	M0	IIB	P	A	FREE	R0	6	3	0.5	0
2035/13	38416	68	M	P.G	OGJ	8×6	NI	NI	A	SIGNET	DT	T2	N1	M0	IIA	P	P	FREE	R0	6	2	0.333	3+
2058/13	44800	41	F	P.G	PYLORO-ANTRUM	8×4	NI	NI	A	TUBULAR	IT	T2	N3	M0	III A	P	A	FREE	R0	5	5	1	1+
2206/13	43517	60	F	P.G	BODY	7×6	NI	NI	A	SIGNET	DT	T3	N3	M0	IIIB	P	P	FREE	R0	12	12	1	2+
2271/13	47568	62	F	P.G	BODY	5.5×3	NI	NI	A	TUBULAR	IT	T3	N1	M0	IIB	P	A	FREE	R0	4	1	0.25	3+
2294/13	49994	58	M	P.G	BODY	6×4	NI	NI	A	TUBULAR	IT	T2	NO	M0	IB	P	A	FREE	R0	2	0	0	1+
2439/13	53101	45	F	P.G	PYLORO-ANTRUM	3×1.2	NI	NI	A	TUBULAR	IT	T2	N0	M0	IB	P	P	FREE	R0	3	0	0	0
2756/13	55510	65	F	P.G	PYLORO-ANTRUM	4×1	NI	NI	P	TUBULAR	IT	T3	N1	M0	IIB	P	A	FREE	R0	2	1	0.5	1+
3129/13	62486	65	M	P.G	OGJ	10×9	NI	NI	A	SIGNET	DT	T2	N1	M0	IIA	A	A	FREE	R0	5	1	0.2	0
3254/13	63250	80	F	P.G	BODY	5×4	NI	NI	P	PAPILLARY	IT	T3	N0	M0	IIA	P	A	FREE	R0	3	0	0	0
3469/13	71799	55	F	P.G	BODY	5×3	NI	NI	A	PAPILLARY	IT	T2	NO	M0	IB	P	A	FREE	R0	11	0	0	1+
95/14	2345	44	F	P.G	PYLORO-ANTRUM	4×3.5	NI	NI	P	TUBULAR	IT	T3	N0	M0	IIA	P	A	FREE	R0	11	0	0	0
108/14	4567	70	M	P.G	PYLORO-ANTRUM	3×2.5	NI	NI	A	TUBULAR	IT	T3	N2	M0	III A	A	A	FREE	R0	10	6	0.6	0
384/14	8247	43	M	T.G	PYLORO-ANTRUM	5×3	NI	NI	A	PAPILLARY	IT	T2	N2	M0	IIB	P	P	FREE	R0	5	4	0.8	1+
776/14	11895	63	F	P.G	PYLORO-ANTRUM	4.5×1.5	NI	NI	A	TUBULAR	IT	T2	N0	M0	IB	P	P	FREE	R0	2	0	0	1+
1069/14	17943	67	M	T.G	BODY	5×2.5	NI	NI	A	TUBULAR	IT	T3	N1	M0	IIB	P	P	FREE	R0	3	2	0.666	0
1406/14	24743	55	M	P.G	PYLORO-ANTRUM	3×1.5	NI	NI	A	TUBULAR	IT	T2	N0	M0	IB	P	A	DISTAL	R1	1	0	0	0
1463/14	23709	45	M	P.G	PYLORIC ANTRUM	7×7	NI	NI	A	TUBULAR	IT	T2	N3	M0	III A	P	A	FREE	R0	12	12	1	1+
1530/14	26359	57	M	P.G	PYLORO-ANTRUM	4×2.5	NI	NI	A	TUBULAR	IT	T3	N1	M0	IIB	P	A	FREE	R0	8	2	0.25	1+
1195/14	16787	60	M	P.G	PYLORO-ANTRUM	3.5×2	NI	NI	A	TUBULAR	IT	T3	N2	M0	III A	P	A	FREE	R0	5	5	1	3+
1992/14	33923	57	F	P.G	PYLORO-ANTRUM	7.5×6	NI	NI	P	DIFFUSE	DT	T4	N1	M0	IIIB	P	A	FREE	R0	6	1	0.166	0
2301/14	38331	52	M	P.G	BODY	6×4	NI	NI	P	TUBULAR	IT	T3	N1	M0	IIB	A	A	FREE	R0	6	1	0.166	0
2300/14	38481	45	M	P.G	PYLORO-ANTRUM	6.5×5	NI	NI	A	TUBULAR	IT	T3	N0	M0	IIA	P	A	FREE	R0	0	0	0	0

KEY TO MASTER CHART

Proc – Procedure

Hist. – Histological

LI – Lymphatic invasion

VI – Vascular invasion

PNI – Perineural invasion

LN – Lymph Node status

PG – Partial Gastrectomy

TG – Total Gastrectomy

IT – Intestinal Type

DT – Diffuse Type

G – Grade

T – Tumour depth

P – Present

A – Absent

N – Node

M – Metastasis

NI – Not Involved

I – Involved

RM – Resected Margins

TLN – Total Lymph Nodes

PN – Positive Nodes

LNR – Lymph Node Ratio